

# ANATOMY AND SYSTEMATICS OF THE SUBMARINE-CAVE GASTROPOD *PISULINA* (NERITOPSINA: NERITILIIDAE)

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## ABSTRACT

The soft parts and radula of *Pisulina adamsiana* and *Pisulina maxima* (Gastropoda: Neritopsina) are described for the first time on the basis of specimens collected alive from nine submarine caves of tropical to subtropical Pacific islands. To clarify the systematic position of *Pisulina* within the Neritopsina the fresh- and brackish-water genus *Neritilia* is also examined anatomically. The close relationship between *Pisulina* and *Neritilia* is confirmed by 19 unique, unreversed, possible synapomorphies: (1) an inclined protoconch; (2) spiral ridges on the larval shell; (3) almost perpendicularly arranged prisms of the outer shell layer; (4) open-pit eyes; (5) female flap on the foot with a ciliated neck furrow from the mantle cavity; (6) caeca on the visceral mass and corresponding shell pores; (7) reduced left ctenidium; (8) right hypobranchial gland composed of single-type cells; (9) two complicated pairs of oesophageal glands; (10) another pair of ovoid glands in the oesophageal floor; (11) inner lateral tooth of radula with a largely expanded lobe; (12) elongated outer lateral tooth with an oblique, comb-like blade; (13) a lateromarginal plate; (14) single large aperture of the digestive gland into the stomach; (15) strongly curved rectum on the pallial roof; (16) transverse slit-shaped vaginal opening situated far back from the female opening and anus; (17) absence of independent duct from the seminal receptacle to the albumen gland; (18) very long, club-shaped spermatophore sac extending to the pericardium; and (19) doubled seminal vesicle. *Pisulina* and *Neritilia* constitute an independent family Neritiliidae, together with the Miocene genus *Pisulinella*. The discovery of the open-pit eye in *Pisulina* and *Neritilia*, hitherto unknown in Orthogastropoda, suggests that the genera evolved from an ancestral species that lived in dark submarine caves.

## INTRODUCTION

In recent years the investigation of marine cave molluscs primarily accessible through SCUBA diving has produced interesting questions of taxonomy, biogeography and reproductive biology (Kase & Hayami, 1992; Hayami & Kase, 1993, 1996). A number of new and already named taxa, which include species regarded as 'living fossils' and relatives of deep-sea taxa, have been discovered (e.g. Hayami & Kase, 1992, 1993, 1996; Kase & Hayami, 1992; Geronimo, Allegri, Improta, La Perna, Rosso & Sanfilippo, 1997; Kase & Kano, 1999, 2002). Discovery of live animals previously known only as empty shells has shed new light on molluscan systematics and evolution.

*Pisulina* Nevill & Nevill, 1869 is a genus of Gastropoda living in cryptic habitats of tropical and subtropical shallow marine waters. The snails were known only from empty shells until Kano & Kase (2000b) found them living in totally dark, submarine caves in the Indo-Pacific. The systematic position of the genus has been problematic. Nevill & Nevill (1869) thought it was close to *Teinostoma* Adams & Adams, 1854 (Caenogastropoda). Thiele (1925) was the first author to place *Pisulina* in Neritidae (superorder Neritopsina). Wenz (1938) included *Pisulina* in the subfamily Smaragdiinae of Neritidae, based solely on gross teleoconch shape and this allocation was subsequently followed by Knight, Cox, Keen, Batten, Yochelson & Robertson (1960) and Komatsu (1986). However, Herbert & Kilburn (1991) found that *Pisulina adamsiana* Nevill & Nevill, 1869 differs in protoconch morphology not only from *Teinostoma*, but also from *Smaragdia* Issel, 1869, the type genus of Smaragdiinae. Recent conchological analysis (Kano & Kase, 2000b) indicates that *Pisulina* is close to *Neritilia* Martens, 1879 and should be classified in the same family Neritiliidae Schepman, 1908.

Here we describe the anatomy of the genus *Pisulina* for the first time. The description is based on *Pisulina adamsiana* and *Pisulina maxima* Kano & Kase, 2000, the only species so far collected alive, and *Neritilia* is examined for comparison.

## MATERIAL AND METHODS

Thirty individuals of *Pisulina adamsiana* and 15 of *Pisulina maxima* were examined anatomically. The former species was collected from submarine caves on Sipadan Island in Malaysia (seven specimens), Hawai'i Island (17 specimens), Saipan in the Northern Mariana Islands (one specimen), Palau (three specimens) and Christmas Island in the Indian Ocean (two specimens). *Pisulina maxima* was collected from Sipadan Island (13 specimens) and Palau (two specimens). Living animals of both species were obtained from the walls inside limestone and lava caves at depths ranging from 6 to 40 m, picked up by hand or by brushing the under-surface of coral rubble on the bottom (see Kano & Kase, 2000b, for locality details).

The external morphology of the living animals was observed under a binocular dissecting microscope. The animals were then relaxed in 7.5% magnesium chloride, fixed in 10% formalin for 24 h and then preserved in 75% ethanol. The preserved animals, extracted from the shells, were also observed under the binocular microscope and dissected after staining with methylene blue or haematoxylin. Materials for histological study were embedded in paraffin and sectioned at 6–10 µm, and the serial sections stained with haematoxylin-eosin. For SEM observation of soft parts, animals were gradually dehydrated, transferred to *n*-butyl alcohol and dried with a freeze-drier. Radulae were removed from the buccal mass, soaked in sodium hypochlorite for several minutes, then washed, mounted and dried. All specimens used in this study are deposited in the National Science

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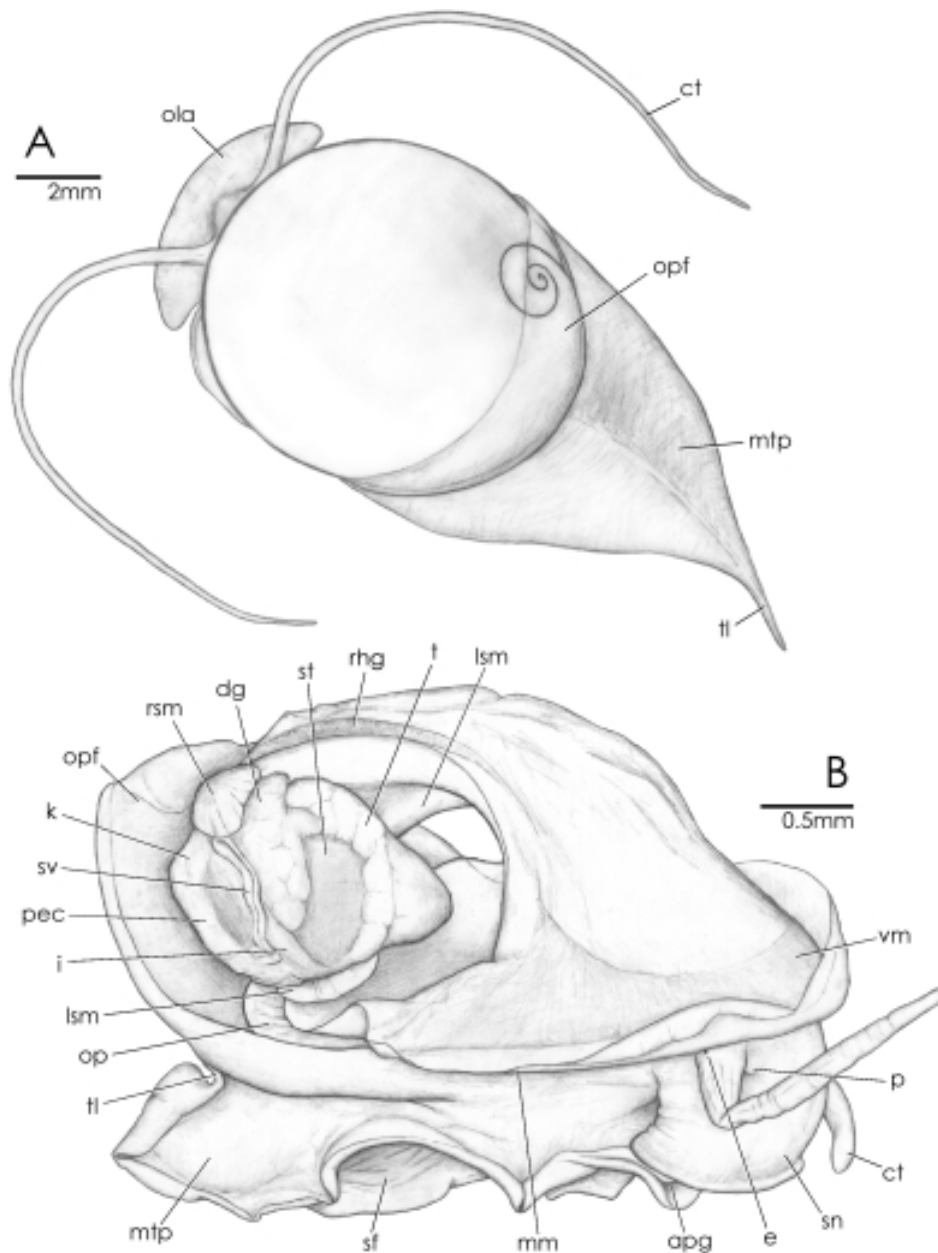
Museum, Tokyo. Anatomical terminology generally follows Fretter (1965), Fretter & Graham (1994) and Sasaki (1998). The description applies to both *P. adamsiana* and *P. maxima*, except where noted otherwise.

For comparative purposes, we anatomically examined the following taxa: six species of *Neritilia* (Neritiliidae), including *Neritilia rubida* (Pease, 1865), *Neritilia succinea* (Récluz, 1841), *Neritilia mimotoi* Kano, Sasaki & Ishikawa, 2001 and three unidentified species from the western Pacific islands; *Smaragdia rangiana* (Récluz, 1841) and *Smaragdia souverbiana* (Montrouzier, 1863) of the subfamily Smaragdiinae of the Neritidae, both collected from Okinawa, Japan.

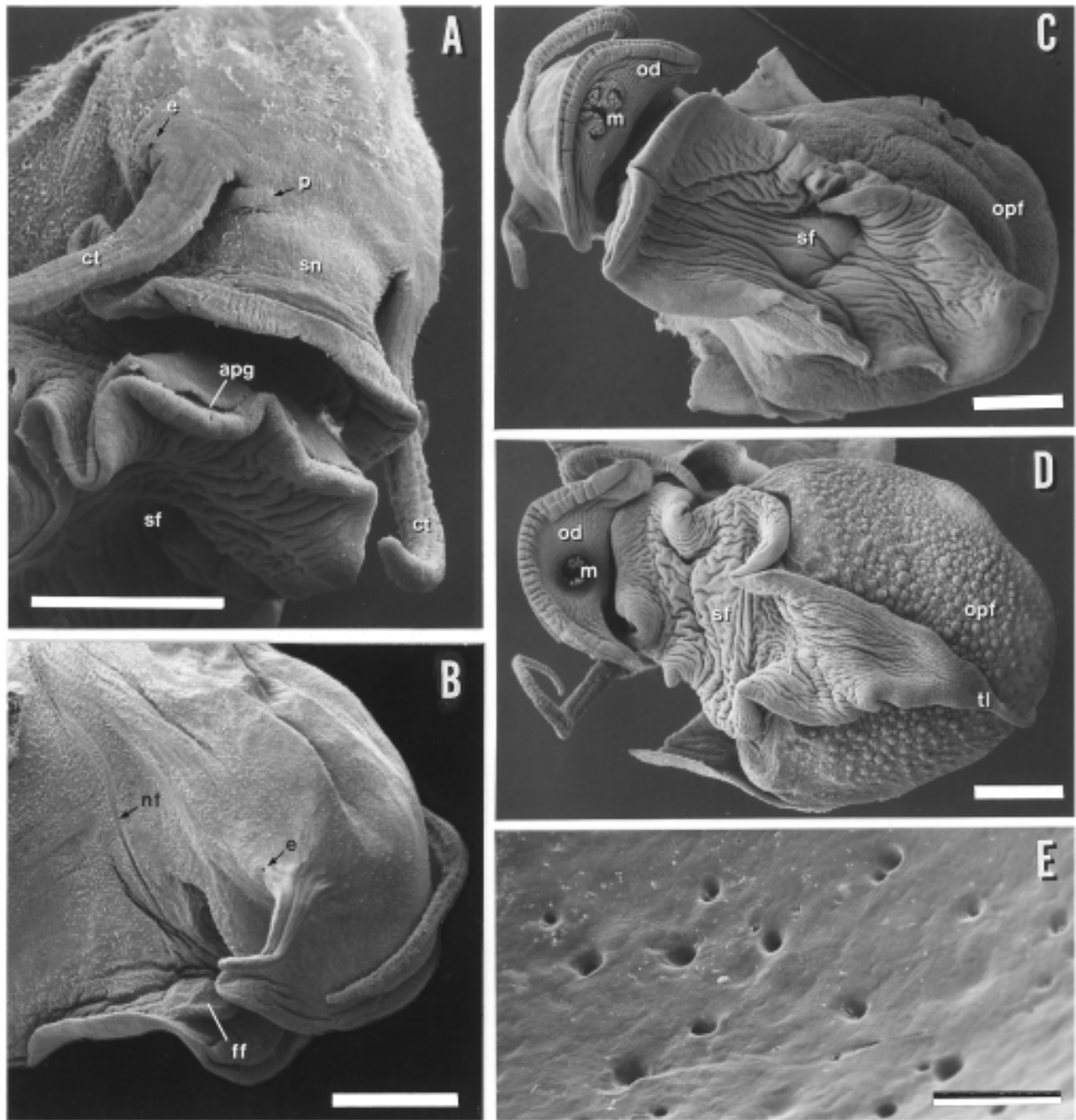
## RESULTS

### External structures

The head has a pair of cephalic tentacles (ct) and a massive snout (sn); its foot (f) has a well-developed metapodium (mtp; Figs 1, 2A–D). The skin is translucent white in colour and lacks any pigmentation throughout; the pinkish-brown buccal mass can be seen through the head wall. The right and left shell muscles (rsm, lsm) extend greatly into the depths of the shell (Fig. 1B). The visceral mass is a small globular hump and does not coil spirally.



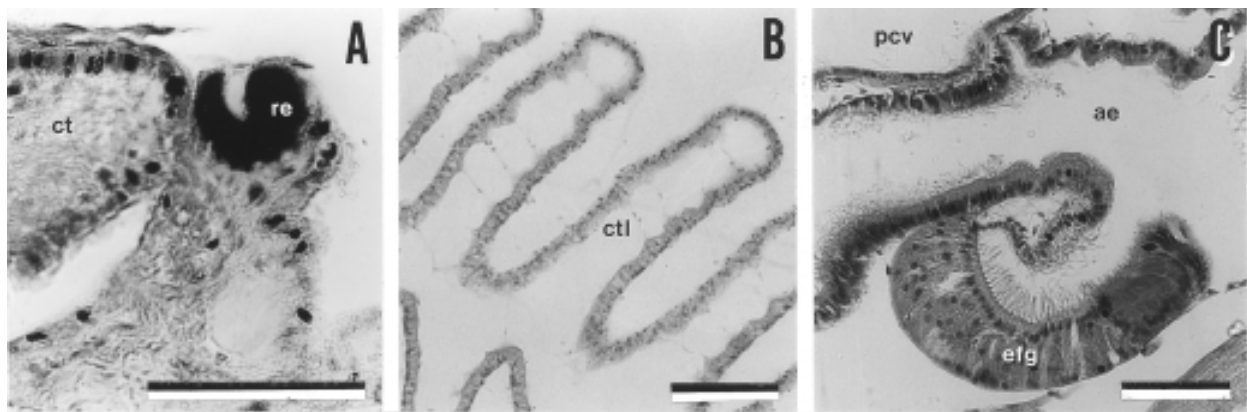
**Figure 1.** Animals of *Pisulina*. **A.** Living animal of *P. maxima* from Palau, dorsal view. **B.** Male specimen of *P. adamsiana* from Sipadan Island (Malaysia), removed from shell, viewed from right side. Abbreviations: apg, opening of anterior pedal gland; ct, cephalic tentacle; dg, digestive gland; e, eye; i, intestine; k, kidney; lsm, left shell muscle; mm, mantle margin; mtp, metapodium; ola, oral lappet; op, operculum; opf, opercular fold of metapodium; p, penis; pec, pericardial cavity; rhg, right hypobranchial gland; rsm, right shell muscle; sf, sole of foot; sn, snout; st, stomach; sv, seminal vesicle; t, testis; tl, tail; vm, vascularized area of mantle skirt.



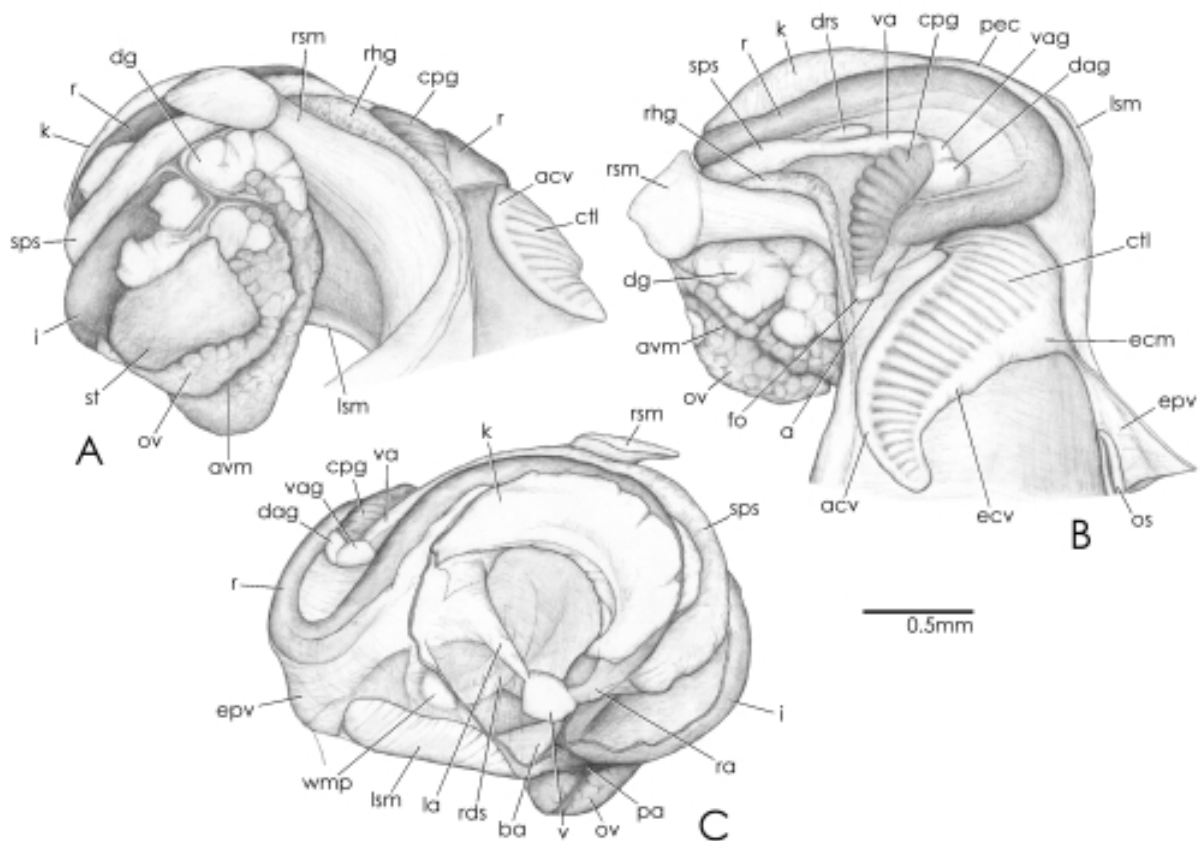
**Figure 2.** SEM photographs of *Pisulina adamsiana* (A–C, E) and *Pisulina maxima* (D), all from Sipadan Island (Malaysia) except E from Hawai'i Island. Mantles removed. **A.** Head region of male from anteroventral side. Scale bar = 100  $\mu\text{m}$ . Note vestigial eye and small penis. **B.** Head region of female showing female flap (presumably an ovipositor) and neck furrow extending to it. Scale bar = 500  $\mu\text{m}$ . **C, D.** Ventral views of animals of two species. Scale bars = 500  $\mu\text{m}$ . Note presence (D) or absence (C) of warts on opercular fold of metapodium. **E.** Micropores on internal surface of shell; these correspond with filamentous caeca on the visceral mass. Scale bar = 50  $\mu\text{m}$ . Abbreviations: apg, opening of anterior pedal gland; ct, cephalic tentacle; e, eye; ff, female flap; m, mouth; nf, neck furrow to female flap; od, oral disk; opf, opercular fold of metapodium; p, penis; sf, sole of foot; sn, snout; tl, tail.

The cephalic tentacles are exceptionally long for the Neritopsina, approximately 1.5 times longer than the shell diameter (Fig. 1A). Their surfaces are devoid of micropapillae and sculptured only with indistinct longitudinal grooves. The eye (e) is very small (especially in *Pisulina maxima*), located at the outer base of each tentacle, without a peduncle (Figs 1B, 2A, B). It is an open vesicle lined with pigmented retina (Fig. 3A: re), and lacks both a cornea and vitreous body (or lens). Cephalic lappets are absent. The snout expands ventrolaterally to form oral lappets (ola). The oral disk (od) is semicircular in shape, with a ventral mouth (m); neither the oral disc nor the mouth opening is cuticularized. As the mouth opens and the tip of the

odontophore advances papillated projections on the lateral sides of the inner lip are exposed (Fig. 2C, D). The neck lobes are weak, but distinct in *P. maxima*, originating at the base of the cephalic tentacle on each side; they are totally absent in *Pisulina adamsiana*. The foot is long and broad, with a ciliated sole throughout. The anterior end is truncated, with a deep transverse groove where an anterior pedal mucous gland opens (apg). There are no epipodial tentacles. The metapodium (posterior half of the whole foot) has a weak dorsomedial ridge and is pointed posteriorly to form a slender tail (tl). The extended opercular fold of the metapodium (opf) covers the shell posteriorly and wraps the operculum completely (Fig. 1A). While



**Figure 3.** Histology of *Pisulina adamsiana* (A, C) and *Pisulina maxima* (B). Haematoxylin and eosin. **A.** Horizontal section through basal region of cephalic tentacle, showing open-pit eye without vitreous body. Scale bar = 50  $\mu\text{m}$ . **B.** Longitudinal section through midline of ctenidium. Scale bar = 100  $\mu\text{m}$ . **C.** Longitudinal section through oesophageal floor gland, buccal cavity toward left. Scale bar = 50  $\mu\text{m}$ . Abbreviations: ae, anterior oesophagus; ct, cephalic tentacle; ctl, ctenidial leaflet; efg, oesophageal floor gland; pcv, pallial cavity; re, retina.



**Figure 4.** Visceral mass and pallial cavity of female *Pisulina adamsiana*. Shell and mantle removed. **A.** From slightly ventral right side. **B.** Anterodorsal view. **C.** From posterior side; radular sac is seen through transparent pericardial wall. Abbreviations: a, anus; acv, afferent ctenidial vein; avm, artery around visceral mass; ba, bulbous aorta; cpg, capsule gland; ctf, ctenidial leaflet; dag, dorsal albumen gland; dg, digestive gland; drs, duct to seminal receptacle; ecm, efferent ctenidial membrane; ecv, efferent ctenidial vein; epv, efferent pallial vein; fo, female opening of gonoduct; i, intestine; k, kidney; la, left auricle; lsm, left shell muscle; os, osphradium; ov, ovary; pec, pericardial cavity; r, rectum; ra, right auricle; rds, radular sac; rhg, right hypobranchial gland; rsm, right shell muscle; sps, spermatophore sac; st, stomach; sv, seminal vesicle; t, testis; v, ventricle; va, vagina; vag, ventral albumen gland; wmp, white mass on pericardial wall.

the outer surface of the lobe is warty in *P. maxima*, it is relatively smooth in *P. adamsiana* (Fig. 2C, D). The warts of *P. maxima* consist of glandular, eosin-stained cells. The mantle margin (mm) is simple with an outer mantle groove and is devoid of microtentacles. The mantle expands posteriorly as a thin pad between the shell and operculum (op), and lacks the 'opercular gland' described by Bourne (1909: 850) for *Nerita*. The shell muscles are long, slender and not clearly subdivided into

bundles. In the visceral region, the gonad, digestive gland (dg), stomach (st), intestine (i), kidney (k) and pericardium (pec) are visible through the transparent mantle (Figs 1B, 4). Connective tissue is scarce beneath the mantle of the visceral mass and between the gonad and digestive gland. The mantle covering the visceral mass bears numerous filamentous caeca, which extend into the micropores in the internal surface of the shell (Fig. 2E). The caeca and micropores are unbranched and

## ANATOMY AND SYSTEMATICS OF *PISULINA*

**Table 1.** Maximum shell diameters of dissected specimens of *Pisulina*, showing size difference between sexes. 5 specimens of *Pisulina maxima* were not mature and their sex was not determined; note that largest (presumably female) juvenile is larger than smallest mature male.

	<i>Pisulina adamsiana</i>				<i>Pisulina maxima</i>			
	<i>n</i>	Range	Mean	SD	<i>n</i>	Range	Mean	SD
Females	11	3.4–7.5	4.78	1.11	3	4.9–9.4	6.83	2.32
Males	19	3.1–4.6	3.65	0.47	7	3.7–9.0	4.96	1.83
Juveniles	0	–	–	–	5	2.9–4.1	3.54	0.45
Total	30	3.2–5.6	3.82	0.63	15	2.9–9.4	4.86	1.93

fairly long, but never penetrate the whole thickness of the shell. The micropores are 3–12 µm in diameter.

The sexes are separate and there is a size difference between them; the mean maximum shell diameter of females is 1.3–1.4 times greater than that of males (Table 1). The male has a very small projection (p) at the left of the base of the right cephalic tentacle (Figs 1B, 2A). This putative rudimentary penis is simple, with no lateral groove. A ciliated groove from the genital opening to the penis (described for some neritids and *Titiscania* by Houston, 1990) was not found even by SEM observations. The female has a flap (ff) on the dorsal surface of the foot at the right side, posterior to the opening of the anterior pedal mucous gland (Fig. 2B). A ciliated neck furrow (nf) originates in the depths of the mantle cavity and extends along the right shell muscle to an anterior transverse cleft in the female flap.

### *Pallial complex*

The mantle cavity is remarkably deep and contains the left ctenidium, left osphradium (os), rectum (r) and closed genital duct. There is no 'right vestigial gill' (Fretter, 1965), as is present in the Neritidae.

The ctenidium is bipectinate and short. It is less than half the length of the mantle cavity depth so that the tip does not stick out from the cavity (Fig. 4B). The unattached end is about three-fifths of the whole ctenidial length. The ctenidial axis runs transversely, and is supported by afferent and efferent membranes (acm, ecm); the former attaches to the rectum and the latter arises from the pallial wall dorsal to the left shell muscle. The number of ctenidial leaflets (ctl) on each side is up to 26 in *P. adamsiana* and up to 39 in *P. maxima*. Each leaflet is short (Fig. 3B) and lacks any projection of the midline. The skeletal rod is absent. The osphradium consists of two ciliated, parallel ridges with a median furrow and is positioned anterior to the attachment of the efferent ctenidial membrane along the left shell muscle (Fig. 4B).

On the right of the mantle cavity a strip of a brownish-yellow hypobranchial gland (rhg) lies between the right shell muscle and genital duct, extending from the very back of the mantle cavity anteriorly to the mouth of the cavity (Figs 1B, 4A, B). The gland differs histologically from that of neritids despite the similar positions and arrangements; it is devoid of supporting cells and composed of single-type, eosin-staining columnar cells. The gland was found in *Neritilia succinea* by Andrews (1937), who assumed that the secretion aids the passage of spermatophores from males to females.

### *Digestive system*

**Buccal cavity and oesophagus.** The buccal cavity (bcv) is large, supported by two pairs of cartilages (bc) and accompanied by a deep sublingual pouch (slp) underlying the odontophore (Fig. 5A, E). A small median cartilage (cp) supports the posterior wall of the pouch and is pulled by a pair of protractor muscles (mps).

The pouch projects laterally to form a small, sac-like, thin-walled, sublingual gland (sg) on each side near the buccal cartilage. The wall of the buccal cavity is cuticularized throughout and longitudinally folded near the mouth. The transverse buccal fold (bf) on the roof is pulled by dorsal retractor and lateral extensor muscles (drf, lef). The buccal cavity is limited posteriorly by the dorsal lip of the radular diverticulum, which forms a mobile flap (the oesophageal valve).

The succeeding, dorsoventrally depressed anterior oesophagus (ae) bears anterior and posterior pairs of oesophageal glands (aeg, peg). The anterior half of each anterior gland opens medially into the oesophagus along its length (Fig. 5C); the anterior tip of the gland, however, projects forward as a blind pouch overlying the buccal cavity. The posterior half of the gland extends as a long sac overlying the anterior oesophagus and projects posteriorly as far as the mid-oesophagus (Fig. 5D); it connects with the anterior half of the gland through a restricted opening. At this junction, the anterior glands also expand ventrally to develop large pouches, which cover the posterior odontophoral cartilages posteroventrally and also communicate with the posterior oesophageal glands (Fig. 5B). The posterior glands (peg) open laterally to the anterior oesophagus with nearly their entire length posterior to the openings of the anterior glands. They expand ventrally to meet each other and to form a long trough in which the radular sac runs back beneath the oesophagus (Fig. 5D). The posterior tips of the posterior glands are shortly free from the trunk of the oesophagus under the tips of the anterior gland. Both pairs of glands are only very weakly folded interiorly. No definite salivary glands are present.

The anterior oesophagus bears another pair of glands that are markedly different morphologically and histologically from the oesophageal glands described above. These glands (oesophageal floor glands; efg) are ovoid and stout outgrowths in the floor posterior to the valve (Fig. 5B). Each outgrowth comprises two histologically distinct parts: the anterior two-thirds is composed of columnar, secretory, ciliated cells, while the remaining one-third of single-type, columnar, secretory cells stained heavily with haematoxylin (Fig. 3C). The lumina of the glands opened posterodorsally; they are continuous with grooves running on each side of a median longitudinal ridge. The ridge extends to the posterior end of the anterior oesophagus.

The mid-oesophagus (me) narrows as it runs from anterior to posterior oesophagus (pe); its inner surface is sculptured with longitudinal grooves. The posterior oesophagus extends back to the right side of the stomach, opening into the latter through a narrow constriction (Fig. 7A, B).

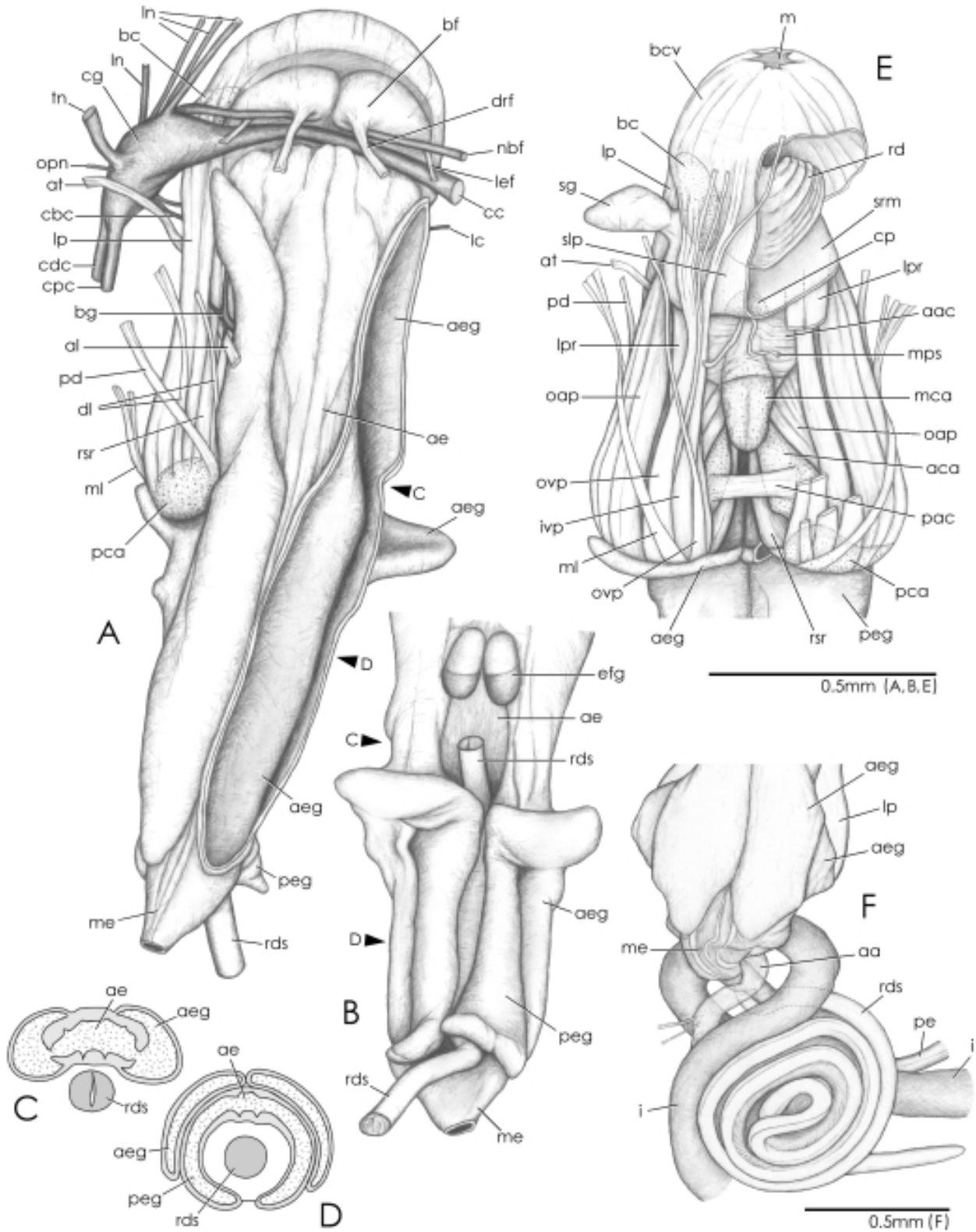
**Buccal mass.** The features related to the buccal mass of *Pisulina* (Figs 5A, E, 6B) are nearly identical to those of other Neritopsina described by Fretter (1965) and Sasaki (1998). The internal skeleton of the buccal mass consists of anterior, posterior and median cartilages. The long anterior cartilages (aca) are beak-shaped anteriorly, and truncated and abutting on the posterior



cartilages (pca) posteriorly (Fig. 6B). The two anterior cartilages are connected to each other ventrally by anterior and posterior approximators; the broad anterior approximator (aac) is inserted on their anterior halves and the narrow posterior approximator (pac) is inserted near their posterior ends. The unpaired median cartilage (mca) lying between the anterior cartilages attaches anteriorly to the dorsal surface of the

anterior approximator for two-thirds its length. The anterior and posterior pairs of cartilages are connected ventrally by the outer approximator muscles (oap). The posterior cartilages are not connected to each other.

The odontophore is controlled by muscles originated from the body wall: lateral protractors (lp), outer and inner ventral protractors (ovp, ivp), median and dorsal levators (ml, dl), pos-

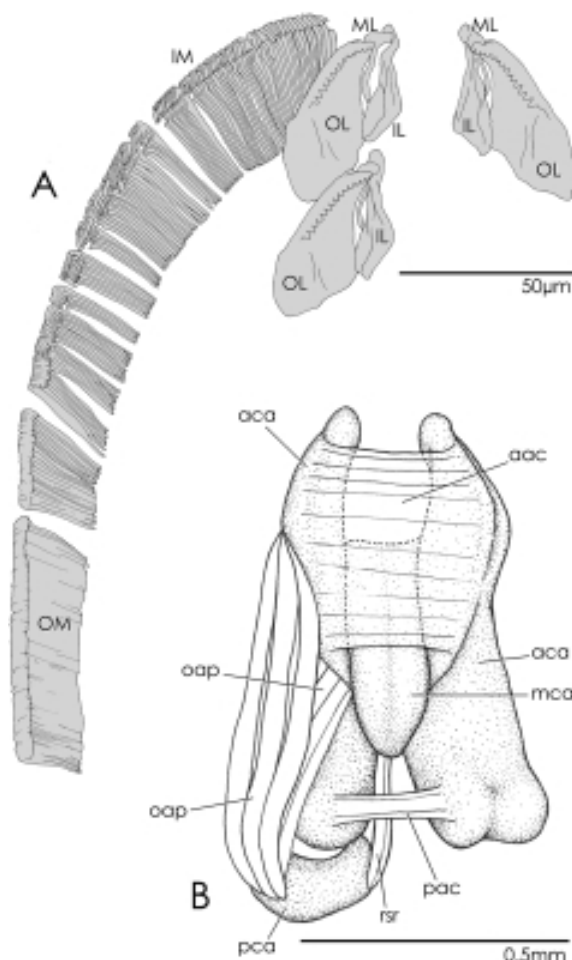


terior depressors (pd; those inserted on the posterior odontophoral cartilages), anterior levators (al) and anterior tensors (at; those inserted on the anterior cartilages). The subradular membrane (srm) is controlled by the retractors (rsr) and lateral protractors (lpr), both inserted on the posterior cartilages.

**Radula:** The radular sac is very long and more than twice as long as the shell diameter (Fig. 5F). The number of transverse rows is over 190 in *P. maxima*. From the buccal mass the sac extends back through the trough of the posterior oesophageal glands, and then coils spirally beneath the floor of the mantle cavity and dorsal to the posterior oesophagus. The sac coils four times and the coiling direction usually changes from clockwise to counter clockwise in the third loop (sometimes the change occurs in the second loop). The succeeding short and straight, tooth-secreting portion enters the visceral mass immediately behind the kidney.

The radula is rhipidoglossate, bilaterally symmetrical, lacks a central tooth and has the formula  $\infty-3-0-3-\infty$  (Figs 6A, 8; Kano & Kase, 2000b: fig. 12). The inner lateral tooth (IL) is large, thin and longitudinally elongate (Fig. 8A–C). The broad, blade-like cusp is steeply oblique or nearly perpendicular to the transverse axis of the radula. The posterior part of the tooth expands greatly as a lobe and overlies the base of the adjacent median lateral tooth. The outer margin of the lobe is strongly reflected inwardly. The most anterior portion of this tooth (identified by Baker, 1923 as a separate, second lateral tooth in *Neritilia rubida*) projects and is continuous to the outer margin of the lobe. The median lateral tooth (ML) is small, and has a robust quadrangular cusp and a thin shaft (Fig. 8B). The outer lateral tooth (OL) is large and very oblique. The reflected anterior blade has many conspicuous cusps, which decrease in size toward both ends of the reflection; the number of cusps is 10–13 in *P. adamsiana* (Fig. 8A, B) and 15 in *P. maxima* (Fig. 8C). The innermost end of the lateral tooth projects and fits into the concavity of the shaft just below the cusp of the median lateral. The posterior portion forms a thin lobe that largely covers the base of the inner marginal teeth. These features of the lateral field are very similar to those of *Neritilia* figured by Baker (1923).

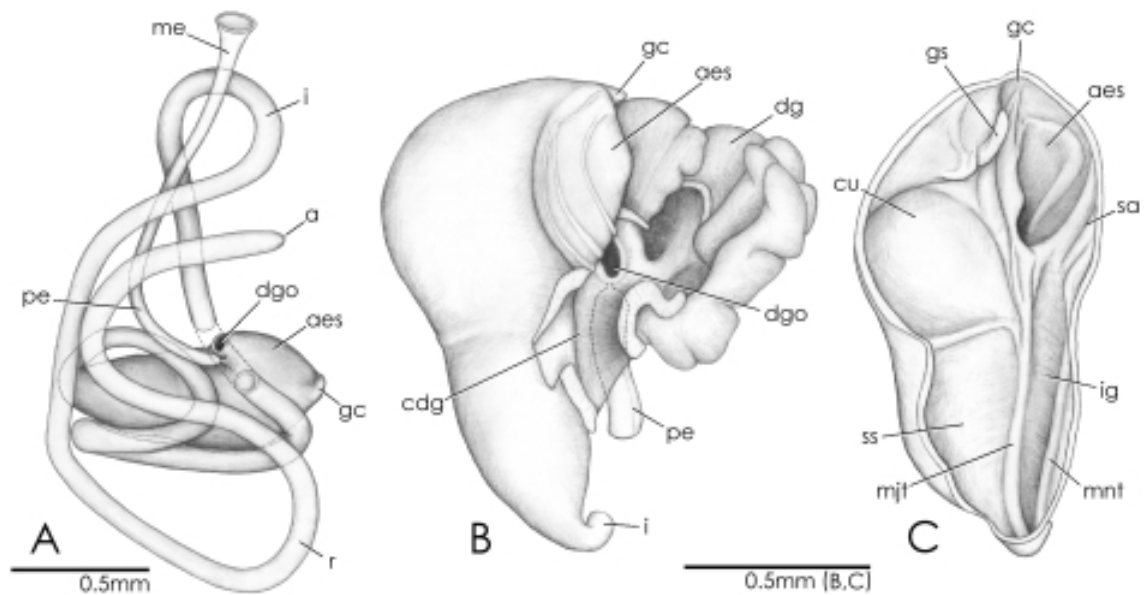
Between the outer lateral and innermost (first) marginal teeth is a thin, short, lateromarginal plate (LMP) without any conspicuous ornamentation (Fig. 8D). There are over 170 marginals (M) in either side of each transverse row. The inner marginals are longitudinally flattened, slightly curved and basally constricted. Each tooth bears overhanging cusps of various sizes along the distal portion and a thin, triangular prominence below the cusps. Ten inner marginals in *P. adamsiana* and about 22 in *P. maxima* have two cusps each; a large, stout spike-like cusp at the front and a small cusp at the back. The number of cusps in succeeding teeth increases toward the outer marginal teeth and the cusps become larger toward the front of each tooth (Fig. 8D–G). The outer marginals are narrower than the inner



**Figure 6.** Diagrammatic drawings of digestive system of *Pisulina*. **A.** Central and lateral fields and left marginal field of one transverse row of radula. Left lateral field of next anterior row is also included to show relationship. **B.** Buccal cartilages and approximators, ventral view. Posterior cartilage and some muscles are removed on right side. Abbreviations: aac, anterior approximator of cartilages; aca, anterior cartilage of odontophore; IL, inner lateral tooth; IM, inner marginal teeth; LMP, lateromarginal plate; mca, median cartilage of odontophore; ML, median lateral tooth; oap, outer approximator of cartilages; OL, outer lateral tooth; OM, outer marginal tooth; pac, posterior approximator of cartilages; pca, posterior cartilage of odontophore; rsr, retractor of subradular membrane.

marginals, with a glove- or spoon-like distal portion that bears many cusps and a prominence. In this portion, the cusps are nearly the same size, and the prominence is relatively long and acute at the inner side of the teeth. The serrated margin of the outermost marginals is made up of more than 20-min cusps.

**Figure 5.** Digestive system of *Pisulina adamsiana*. Odontophore is in protruded condition in **A** to **E**. **A.** Buccal mass and anterior oesophagus, dorsal view. On right, buccal mass, cerebral ganglion and dorsal wall of anterior oesophageal gland are cut away. Sublingual glands are omitted. **B.** Ventral view of anterior oesophagus. Buccal mass is removed, and both ends of radular sac are cut away to show ventral outgrowths of oesophageal floor glands. Posterior one-third of floor glands are stained with haematoxylin. **C, D.** Diagrammatic transverse sections of anterior oesophagus and radular sac, taken at positions indicated in **A** and **B** by arrowheads. Not to scale. **E.** Buccal mass and buccal cavity, ventral view. Part of buccal wall, ventral pouch of anterior oesophageal gland and some muscles are cut away on right side. Nervous system is not drawn. **F.** Dorsal view of oesophagus and loops of radular sac. Abbreviations: aa, anterior aorta; aac, anterior approximator of cartilages; aca, anterior cartilage of odontophore; ae, anterior oesophagus; aeg, anterior oesophageal gland; al, anterior levator of odontophore; at, anterior tensor of odontophore; bc, buccal cartilage; bcv, buccal cavity; bf, buccal fold; bg, buccal ganglion; cbc, cerebrobuccal connective; cc, cerebral commissure; cdc, cerebropedal connective; cg, cerebral ganglion; cp, cartilage of sublingual pouch; cpc, cerebropleural connective; dl, dorsal levator of odontophore; drf, dorsal retractor of buccal fold; efg, oesophageal floor gland; i, intestine; ivp, inner ventral protractor of cartilages; lc, labial commissure; lef, lateral extensor of buccal fold; ln, labial nerve; lp, lateral protractor of odontophore; lpr, lateral protractor of subradular membrane; m, mouth; mca, median cartilage of odontophore; me, mid-oesophagus; ml, median levator of odontophore; mps, median protractor of sublingual pouch; nbf, nerve to buccal fold; oap, outer approximator of cartilages; opn, optic nerve; ovp, outer ventral protractor of odontophore; pac, posterior approximator of cartilages; pca, posterior cartilage of odontophore; pd, posterior depressor of odontophore; pe, posterior oesophagus; peg, posterior oesophageal gland; rd, radula; rds, radular sac; rsr, retractor of subradular membrane; sg, sublingual gland; slp, sublingual pouch; srm, subradular membrane; tn, tentacular nerve.



**Figure 7.** Digestive system of *Pisulina adamsiana*. **A.** Configuration of alimentary tract, dorsal view. Buccal cavity and anterior oesophagus are omitted and part of intestine is cut away to show orifice of digestive gland to stomach. **B.** Anteroventral view of stomach and digestive gland. Mouth and anus are toward right. Ventral acini of digestive gland are cut away to show internal cavity of gland. Part of posterior oesophagus is seen by transparency. **C.** Internal structure of stomach, opened by posterior longitudinal incision. Abbreviations: a, anus; aes, anterior extension of stomach; cdg, internal cavity of digestive gland; cu, cuticularized area of stomach; dg, digestive gland; dgo, orifice of digestive gland to stomach; gc, gastric caecum; gs, gastric shield; i, intestine; ig, intestinal groove; me, mid-oesophagus; mjt, major typhlosole; mnt, minor typhlosole; pe, posterior oesophagus; r, rectum; sa, sorting area; ss, style sac.

**Stomach:** The pear-shaped stomach (st) lies across the middle of the visceral hump posterior to the gonoduct; its wider, right-hand side, which bears a small gastric caecum (gc), can be seen from the outside through the body wall (Figs 1B, 4A). The digestive gland lies dorsal to the stomach, is beige in colour and slightly paler than the ovary, and consists of relatively large acini (Fig. 4A, B). The structure of the stomach is almost identical to that of other neritopsines apart from the number and position of digestive gland openings. In *Pisulina*, the acini of the digestive gland are united and form an internal cavity (cdg), which communicates with the stomach by one large orifice (dgo) near the opening of the posterior oesophagus (Fig. 7A, B).

The anterior extension (aes) of the right-hand part of the stomach, where the oesophagus and the cavity of the digestive gland open, has three external and one internal ridges. The inner, dorsal wall of the stomach is covered with a thick cuticle (cu) that forms a weak gastric shield (gs) on the right (Fig. 7C). The food-sorting area (sa) is composed of a few, alternating ridges and grooves on the ventral wall. One of these grooves, originating at the gastric caecum and passing to the left, is the intestinal groove (ig). In the left, tapering part of the lumen of the stomach, the intestinal groove is separated from the style sac (ss) by major (mjt) and minor (mnt) typhlosoles, which originate at the gastric caecum and sorting area, respectively. The stomach contents consist mainly of sponge spicules, sand grains and other undigested food remains.

**Intestine and rectum:** The intestine loops in a complicated way (Fig. 7A: i). It leaves the left end of the stomach with a sharp curve to the right. Halfway across the stomach it curves left, returning to near its point of origin. The intestine then passes again to the right and runs beneath the mantle to the right limit of the visceral mass. From this point it extends anteriorly and forms a small loop below the mid-oesophagus (Fig. 5F). It then proceeds posteriorly to the left of the radular loops and continues into the rectum (r). The rectum penetrates the ventricle

in the pericardium and makes a large S-shaped meander from the visceral mass to the pallial roof. The anus (a) opens in the depths of the pallial cavity on the right. The faeces take the shape of simple rods.

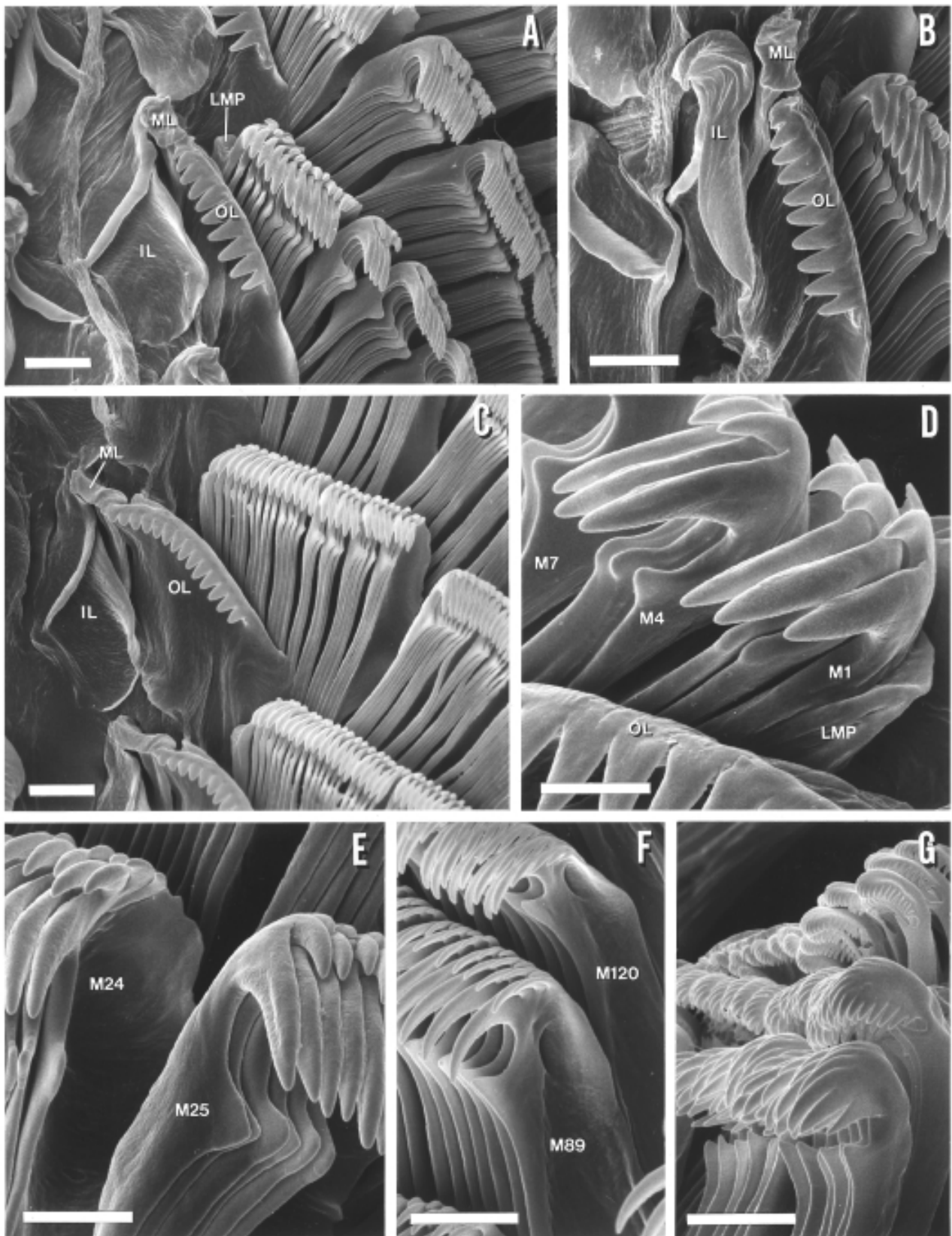
#### *Vascular and excretory system*

**Vascular system:** The pericardial cavity (pec) largely occupies the posterior end of the visceral hump (Fig. 4C). The heart consists of a ventricle (v), and paired left and right auricles (la, ra). The left auricle is attached to the anterior wall of the ventricle and is large, receiving the efferent pallial vein (epv) anteriorly. The right auricle opens in the posterior wall of the ventricle, and is reduced in size and inconspicuous, but it presumably brings part of the purified blood back from the kidney via a vein that partly surrounds the rectum, and spermatophore sac or seminal vesicle. Both the auricles lack the villi of the pericardial gland. On the pericardial wall bordering the mantle cavity dorsal to the left shell muscle is a conspicuous white mass of unknown function (wmp). The cells of the mass are similar to those of the adjacent wall in appearance.

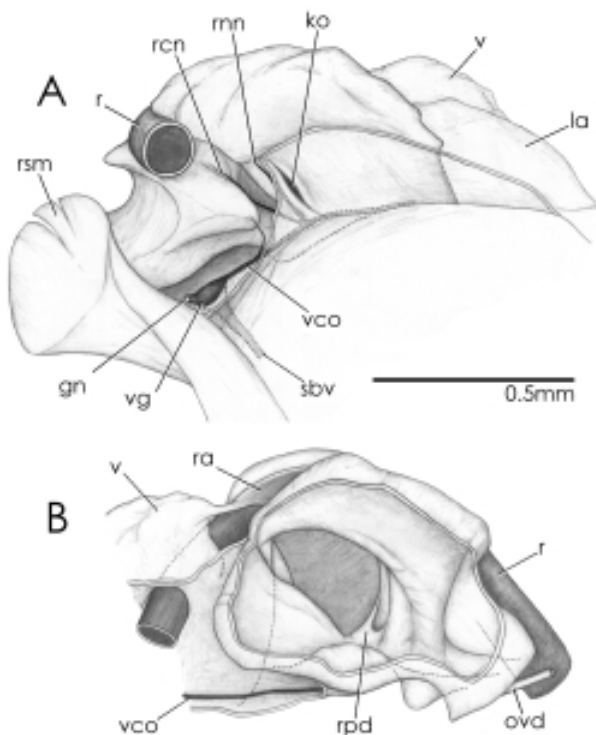
The ventricle is penetrated medially by the rectum. It gives rise to the bulbus aorta (ba) ventrally, which is connected with the anterior and posterior aortae (aa, pa). The anterior aorta extends to the cephalopedal sinus lying ventral to the buccal mass. The posterior aorta transmits blood to the visceral haemocoel, and the main artery passes around the visceral mass on the gonad (Fig. 4A, B: avm).

The anterior, afferent pallial vein largely surrounds the curved rectum and glandular genital duct within the curve. This vein transmits blood from the kidney to the afferent ctenidial vein (acv) and further extends forward along the right shell muscle to the vascularized area of the mantle skirt (Fig. 1B: vm). This vascularized area may serve an important respiratory function, especially as the ctenidium is so poorly developed. The efferent pallial vein running back dorsally along the





**Figure 8.** SEM shots of radulae of *Pisulina adamsiana* from Hawai'i Island (A, B, D–G) and *Pisulina maxima* from Sipadan Island (C). A–C. Lateral and inner marginal teeth; lateral region folded in B. Scale bars = 10  $\mu\text{m}$ . D. Outer lateral tooth, lateromarginal plate and inner (1st–7th) marginals. Scale bar = 5  $\mu\text{m}$ . E. Inner marginals. Lateral sides of 24th and 25th teeth are shown; central field toward left. Scale bar = 5  $\mu\text{m}$ . F. Median marginals. Outer lateral sides of 89th and 120th teeth are seen; central field toward left. Scale bar = 5  $\mu\text{m}$ . G. Outer marginals. Lower, middle and upper rows are around 120–130th, 150–170th and 170–190th teeth, respectively. Central field toward right. Scale bar = 5  $\mu\text{m}$ . Abbreviations: IL, inner lateral tooth; LMP, lateromarginal plate; M, marginal tooth; ML, median lateral tooth; OL, outer lateral tooth. Numbers indicate ordinals of marginal teeth.



**Figure 9.** Kidney and its surrounding structures of *Pisulina adamsiana*. **A.** Frontal view, mantle and pallial gonoduct are removed and rectum is cut away from half its length. The concavity on left of kidney is position of removed spermatophore sac. Nerves pass inside veins; part of these and ventral limit of kidney are seen by transparency. **B.** Ventral view, mantle and ventral wall of kidney are removed. Pallial cavity toward bottom. Abbreviations: gn, genital nerve; ko, kidney opening; la, left auricle; ovd, oviduct; r, rectum; ra, right auricle; rcn, rectal nerve; rmn, renal nerve; rpd, renopericardial duct; rsm, right shell muscle; sbv, suboesophageal part of visceral loop; vg, visceral ganglion; v, ventricle; vco, part of visceral loop.

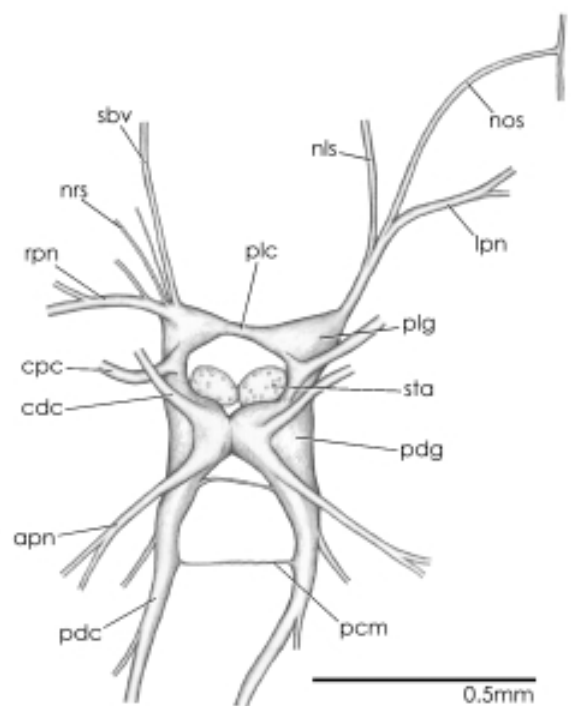
osphradium (Fig. 4B) joins the efferent ctenidial vein (ecv) and then runs into the left auricle.

Erythrocytes were not found in the haemocoel.

**Excretory system.** The single large kidney (k) dorsal to the pericardium and posterior to the mantle cavity partly covers the rectum on the right (Figs 4C and 9). Unlike those of neritids it is not divided into glandular and non-glandular areas anteriorly, and has a vacant space within; its inner surface is glandular throughout and weakly folded only on the dorsal wall. The kidney opening (ko; or uropore) is a vertical slit in the middle of the posterior wall of the mantle cavity and the renal nerve runs alongside this slit to the right (Fig. 9A). The densely ciliated renopericardial duct (rpd), which connects the cavities of the pericardium and kidney, opens to the right of the uropore (Fig. 9B). The nephridial gland was not found in sections.

#### Nervous system

The nervous system of *Pisulina* is hypoathroid. The basic plan agrees with those of other Neritopsina and is identical with that of the phenacolepadid *Phenacolepas omanensis* Biggs, 1973, as described by Fretter (1984). The dorsolaterally positioned large cerebral ganglia (Fig. 5A: cg) are connected with the posteroventrally located pleural (plg) and pedal ganglia (pdg) by long and thick cerebropleural (cpc), and cerebropedal connectives (cdc). The two pedal ganglia are directly connected medially and the pleural ganglia are joined by a short, thick



**Figure 10.** Pleural and pedal ganglia and their associated nerves of *Pisulina adamsiana*, frontal view. Distal parts of nerves are not drawn. Abbreviations: apn, anterior pedal nerve; cdc, cerebropedal connective; cpc, cerebropleural connective; lpn, left pallial nerve; nls, nerve to left shell muscle; nos, nerve to osphradium; nrs, nerve to right shell muscle; pcm, pedal commissure; pdc, pedal cord; pdg, pedal ganglion; plc, pleural commissure; plg, pleural ganglion; rpn, right pallial nerve; sbv, suboesophageal part of visceral loop; sta, statocyst.

pleural commissure (Fig. 10: plc). Though the pleuropedal connectives are very stout and short, the pleural and pedal ganglia are more widely separated than those of any other Neritopsina hitherto reported.

The cerebral ganglia are linked by two commissural nerves: a massive and long cerebral commissure (cc), and a thin labial commissure (lc), running, respectively, above and below the buccal cavity. The ganglia are also linked by fine cerebrobuccal connectives via the buccal ganglia (bg). The cerebrobuccal connectives do not branch off from the labial commissure, but originate directly from the ventral sides of the cerebral ganglia, together with the cerebropleural and cerebropedal connectives. The right and left buccal ganglia, situated posterior to the oesophageal valve, supply nerves both anteriorly and posteriorly. In addition to the commissural and connective nerves, each cerebral ganglion gives off at least seven nerves. A thick tentacular nerve (tn) and an extremely fine optic nerve (opn) arise from the outer lateral side of the ganglion; a relatively thick nerve arises from the inner lateral side of the ganglion, running to the buccal fold (nbf). Four labial nerves (ln) from the anterior part innervate the snout; in these nerves the lowermost one, which innervates the outermost part of oral lappet, is slightly thicker than the others. No nerve to the rudimentary penis was traceable.

The visceral loop is incomplete because of the lack of viscerosupraoesophageal connective. The suboesophageal ganglion is incorporated in the right pleural ganglion. The suboesophageal part of the visceral loop (sbv; or vicro-pleural connective) is slender and extremely long, arising from the right pleural together with the right pallial nerve (rpn) and the nerve

to the right shell muscle (nrs), and running back along the left side of the right shell muscle to the visceral ganglion (vg). The visceral ganglion is situated inside the transverse pallial vein on the right of the body (Fig. 9A) and supplies the genital nerve (gn) in addition to the visceral loop which passes through the vein to the left (vco). This part of the loop is very fine, giving off the rectal nerve (rcn) and renal nerve (rnn). As in the case of the suboesophageal, the supraoesophageal ganglion is incorporated into the left pleural, which innervates the left side of the mantle and pallial wall, left shell muscle, osphradium and single left ctenidium. The left pallial nerve (lpn) is slightly thicker than the other two nerves, one to the shell muscle (nls), and the other (nos) to the osphradium and ctenidium.

The pedal ganglia join together and give off thick pedal cords (pdc) almost in parallel. The pedal cords are linked by at least two, fine pedal commissures (pcm). Also from the pedal ganglia run anterior pedal nerves (apn), which innervate the anterior part of foot, as well as the female flap on the foot. Between the pleuropedal connectives lie the statocysts (sta), which contain many statoconia visible through their translucent walls (Fig. 10).

### Reproductive system

The gonad occupies the anterior half of the visceral mass and covers part of the stomach and digestive gland (Figs 1B, 2A). The closed, glandular pallial gonoduct is attached to the posterior roof of the mantle cavity. This duct extends alongside the rectum, which curves to the left (Fig. 4B, C). The gonopores open far back from the mantle margin.

*Female gonoduct:* The ovary (ov) is beige in colour and consists of acini that are smaller than those of the testis. The oocytes are up to 170  $\mu\text{m}$  in diameter, surrounded by nurse cells. The fine, internally ciliated oviduct (ovd) passes between the kidney and right shell muscle, further runs to the left beneath the vagina, and then enters the albumen gland (Fig. 11A, B). Throughout this course of the oviduct there is no opening to the mantle cavity or pericardium. The albumen gland is creamy in colour, and consists of an anteriorly situated 'dorsal albumen gland' (dag) and a posteriorly situated 'ventral albumen gland' (vag), as in those of neritids and phenacolepadids described by such authors as Fretter (1946), Berry, Lim & Kumar (1973) and Sasaki (1998). The ventral albumen gland, into which the oviduct discharges, is oval in shape. The gland has a particular part adjacent to the dorsal albumen gland, that stains heavily with methylene blue and is identical to the 'clear gland' of *Nerita* described by Bourne (1909). In the dorsal albumen gland the lumen extending from the ventral gland joins the vagina (va) and continues to the lumen of the capsule gland (Fig. 11B, C). The capsule gland (cpg) is dark orange, elongate and extends to the right in an anterior direction. The gland is divided into many transverse sections composed of ciliated epithelial cells and subepithelial secreting cells. The anterior tip of the gland is muscular and leads to the female opening (fo). The opening is located adjacent to the anus, but does not unite with it. The crystal sac (reinforcement sac) observed in the Neritidae (e.g. Andrews, 1937) is absent.

The vagina has an independent aperture (vo) that forms a transverse slit situated far back from the female opening and anus, ventral to the albumen gland (Fig. 11A, B). From its opening, the vagina extends to the right and continues to the spermatophore sac (sps). The sac is long, club-shaped, curved and passes back alongside the rectum on the right, projecting into the pericardium (Fig. 4C). The ciliated cells lining the sac are glandular, but the inner epithelium becomes less glandular and also more muscular toward the point of the divergence of the seminal receptacle (rs). In each specimen examined only

one, simple tube-like spermatophore was stored in the sac and the spermatophore was almost dissolved in some cases. The tip of the spermatophore enters the seminal receptacle through its relatively long, doubled duct. The seminal receptacle is an oval pouch that underlies the vagina and contains the sperm, seen as a white mass. Here, the free sperm are orientated with their tails embedded in the epithelium. Unlike neritids and phenacolepadids, there is no independent duct from the seminal receptacle to the dorsal albumen gland; the sperm from the receptacle enter the albumen gland by way of the vagina. The 'ductus enigmaticus' (Bourne, 1909) is also absent; therefore, the female system of *Pisulina* is diallic (Fig. 12).

The female reproductive glands differ in size and shape between *P. adamsiana* and *P. maxima*; the capsule gland of the latter is more elongated than that of the former (Fig. 11A, B).

*Male gonoduct:* The white testis (t) consists of a small number of acini. The fine vas deferens (vd) from the testis is simple, short and unpigmented; it does not form the highly coiled, black-pigmented 'epididymis', which Bourne (1909) described in neritids. The succeeding portion of the duct, acting as a seminal vesicle (sv), is long and thick with sperm, doubled, but barely coiled and occupies the same position as the spermatophore sac in females (Figs 1B and 11E). The duct is ciliated only near the prostate where it becomes slender again. The prostate (Fig. 11D, E) is divided into the creamy posterior annex gland (ang) and white-coloured anterior prostate gland (pr) as in neritids. The former consists of many follicles and covers part of the latter. The terminal duct from the prostate gland is ciliated and passes anteriorly, opening ventrally as a longitudinal slit (mo) right to the anus.

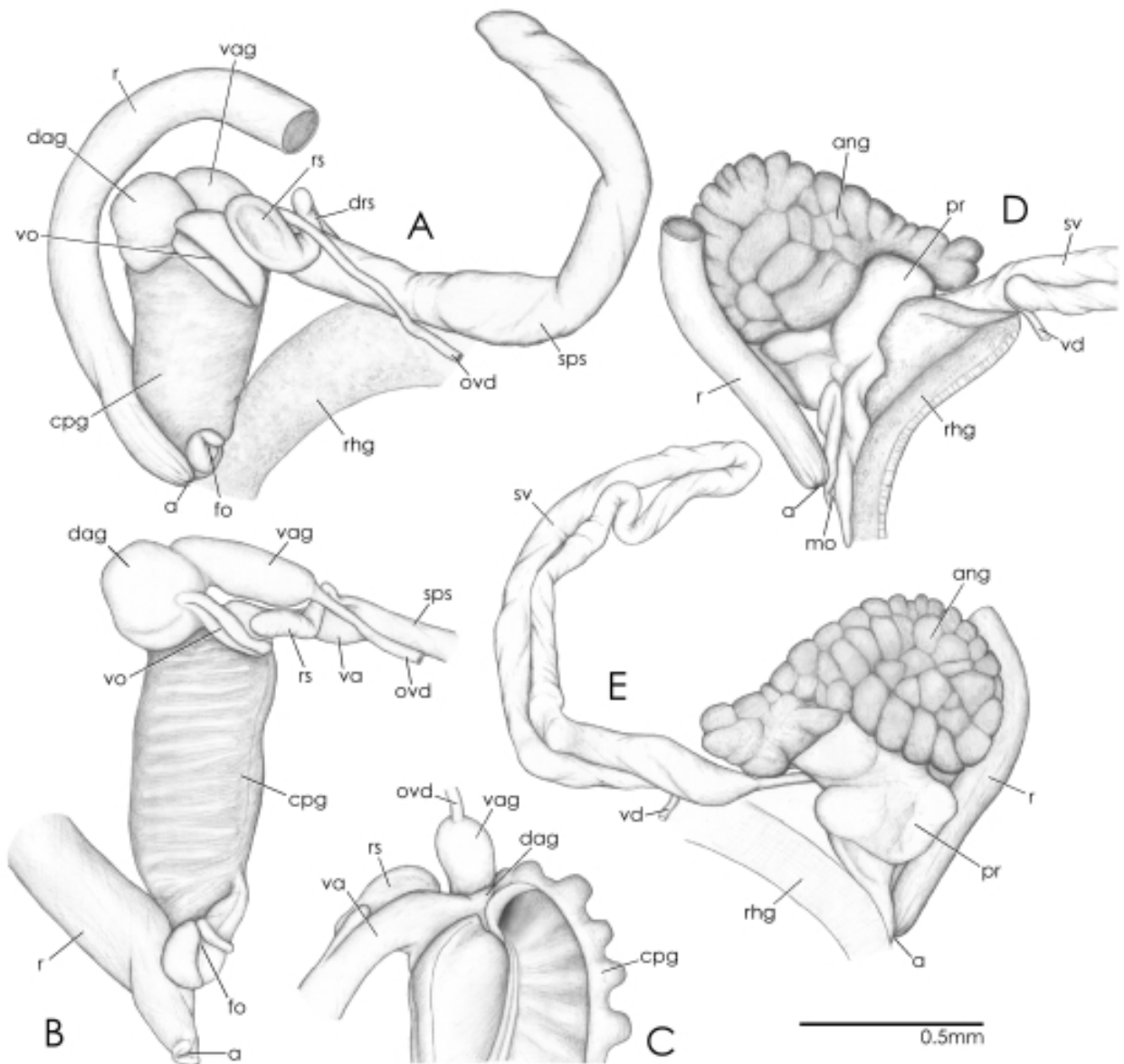
## DISCUSSION

### Character state comparisons with other Neritopsina

*Pisulina* shares a number of features with other genera of Neritopsina, as well as showing features unique to the genus. Kano & Kase (2000b) have demonstrated that *Pisulina* has radular and protoconch features almost identical to those of *Neritilia* (Neritiliidae), rather than to *Smaragdia* (Smaragdiinae of Neritidae), although *Pisulina* had long been assigned to the Smaragdiinae. We discuss here the characteristics of *Pisulina* in comparison with those of other members of the Neritopsina (with emphasis on *Neritilia*) to verify the systematic position of the genus.

The open eye vesicle is one of the most notable features in *Pisulina*. The Neritopsina has hitherto been considered to have a closed eye with a lens (vitreous body), as in fissurellids, caenogastropods and heterobranchs (Starmühlner, 1969: fig. 48; Haszprunar, 1988b: 398; Ponder & Lindberg, 1997: 166; Sasaki, 1998: 133). Figure 13 shows the closed condition of the eyes in *Smaragdia*. However, the eyes of *Pisulina* are represented by extremely small, open vesicles that lack the vitreous body (Fig. 3A). The condition is interpreted most convincingly as degeneration resulting from an adaptation to totally dark, cryptic habitats (as discussed later). Curiously, the same condition of the eyes is also found in *Neritilia* species in rivers and streams (Fig. 14A). These open eyes of *Pisulina* and *Neritilia* further differ from those of neritids in the absence of their peduncles.

Bourne (1909), Andrews (1937), Starmühlner (1969), Fretter (1984), Haynes (1991) and others gave detailed accounts of highly variable male external copulatory organs (penes) of the Neritidae and Phenacolepadidae. The presence of a right cephalic lappet in females of *Olgasolaris* and *Bathynnerita*, and its replacement by a penis exactly at the same position in other neritoideans strongly suggest that the penis of the Neritoidea is modified from the right cephalic lappet (Beck, 1992; Warén & Bouchet, 1993, 2001). The penis is grooved laterally and



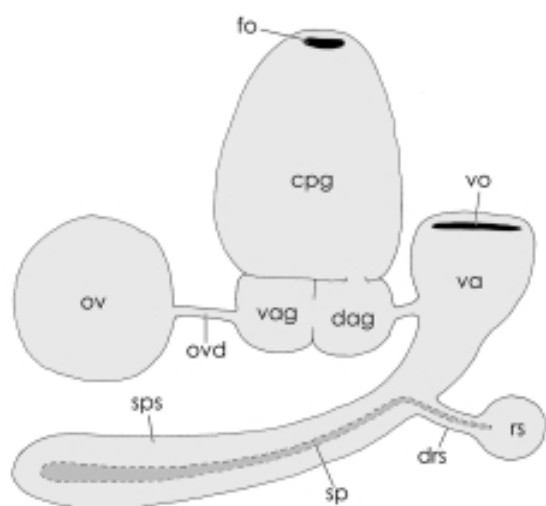
**Figure 11.** Pallial gonoducts of *Pisulina adamsiana* (A, B, D, E) and *Pisulina maxima* (B). A–C. Female gonoducts, ventral (A, B) and left (C) views. Capsule gland is cut along left contact line of dorsal and ventral lobes in C, to expose its lumen connected with that of dorsal albumen gland. D, E. Male gonoduct, dorsal (D) and ventral (E) views. Abbreviations: a, anus; ang, annex gland; cpg, capsule gland; dag, dorsal albumen gland; drs, duct to seminal receptacle; fo, female opening of gonoduct; mo, male opening of gonoduct; ovd, oviduct; pr, prostate gland; r, rectum; rhg, right hypobranchial gland; rs, seminal receptacle; sps, spermatophore sac; sv, seminal vesicle; va, vagina; vag, ventral albumen gland; vd, vas deferens; vo, vaginal opening.

apparently used for the transfer of spermatophores in these two families, while in *Pisulina* it is represented by a very small, rudimentary projection that appears to be functionless (Figs 1B and 2A: p). As observed by Andrews (1937), *Neritilia* has no male copulatory organ in the head region.

Both *Pisulina* and *Neritilia* possess a flap on the dorsal surface of the foot at the right side in females (Figs 2B, 15B: ff). Spawned egg capsules may be transferred via the ciliated groove that originates in the depths of the mantle cavity (near the female opening) and extends along the right shell muscle to the anterior transverse cleft of this female flap. The organ somewhat resembles the ‘female ridge’ (a small projection on the posterior end of the right neck lobe in the Neritidae) of Andrews (1937), who considered its function as guiding the spermatophores during copulation. Presumably homologous with the female ridge is an organ in *Phenacolepas*, which Fretter (1984) described as a ‘cap-shaped enlargement off the anterior

end of the right epipodium’ and regarded as an ovipositor. The female ridge (inclusive of the ‘cap-shaped enlargement’) is present in all the genera of the Neritidae and Phenacolepadidae, except for *Nerita* and vent taxa (Andrews, 1937; Fretter, 1984; Sasaki, 1998: fig. 81). However, the female ridge is most probably not homologous with the female flap of *Pisulina* and *Neritilia*. The two structures are different in position and only the latter is accompanied by a ciliated neck furrow (nf) leading to it. Furthermore, the female ridge is innervated by the right pleural ganglion, while the female flap is innervated by the right pedal ganglion.

*Pisulina* has numerous filamentous mantle caeca of unknown function over the visceral mass. The caeca fit into the microscopic pores in the internal surface of the shell (Fig. 2E). Analogous shell pores have been known widely in the molluscan classes Polyplacophora, Monoplacophora, Gastropoda and Bivalvia (Reindl & Haszprunar, 1996a,b). In Gastropoda the



**Figure 12.** Female reproductive system of *Pisulina*, highly diagrammatic. Abbreviations: cpg, capsule gland; dag, dorsal albumen gland; drs, duct to seminal receptacle; fo, female opening (ootype opening); ov, ovary; ovd, oviduct; rs, seminal receptacle; sp, spermatophore; sps, spermatophore sac; va, vagina; vag, ventral albumen gland; vo, vaginal opening.

pores have been found in Fissurellidae, Peltospiridae (one of the 'hot vent taxa' *sensu* Ponder & Lindberg, 1997), and Phenacolepadidae (for details see Reindl & Haszprunar, 1994). *Shinkailepas* and *Olgasolaris*, both from hydrothermal vents, are the only phenacolepadids with the shell pores (Okutani, Saito & Hashimoto, 1989; Beck, 1992; Reindl & Haszprunar, 1994). Beck (1992) described the caeca of *Olgasolaris*, observing that they reach the periostracum via the shell pores; he hypothesized that they functioned to fix the mantle skirt tightly to the interior shell surface, as well as being secretory or excretory. However, the caeca of *Pisulina* differ significantly from those of *Olgasolaris* in that they never penetrate the whole thickness of the ostracum, suggesting that the caeca in the two groups have different functions. Like those of arcoid and limopsoid bivalves (Reindl & Haszprunar, 1996b), the caeca of *Pisulina* are probably formed secondarily by dissolving the shell; the caeca are present only on the visceral mass and absent near the mantle margin, and the shell pores penetrate not only the complex crossed-lamellar inner layer of the shell (constructed secondarily after absorption of the original layers; Kano & Kase, 2000b), but also the simple crossed-lamellar middle layer (secreted exclusively by the mantle margin). We have found that all species of *Neritilia* have caeca and shell pores identical with those of *Pisulina*.

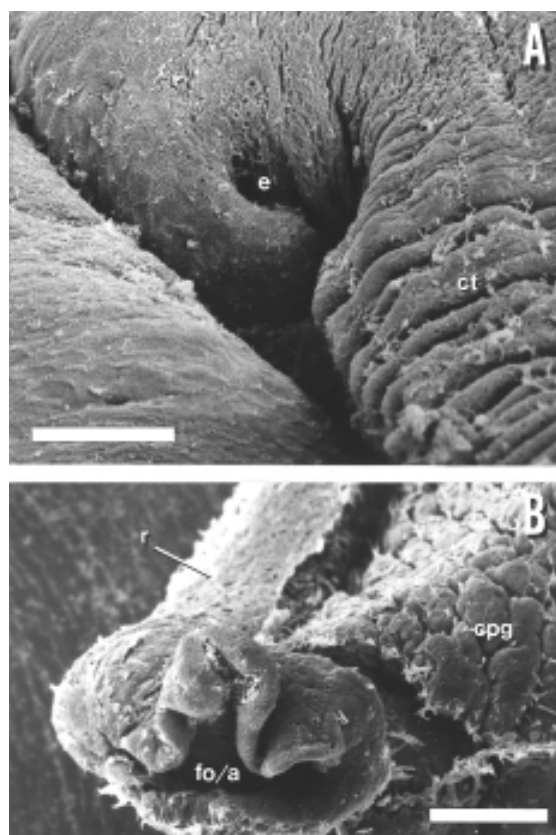
Aquatic members of Neritopsina consistently have a single left, bipectinate ctenidium without skeletal rods. In *Pisulina* and *Neritilia* the ctenidium is remarkably smaller than that in other Neritopsina and the leaflets are very short, so that the ctenidium never projects from the mantle cavity (Figs 3B, 4B, 15B). Comparing similar-sized animals, the number of leaflets is less than 26 along each side of the ctenidium in *Pisulina adamsiana*, while it is over 50 in *Smaragdia rangiana*. In addition, *Pisulina* and *Neritilia* lack the 'vestigial right gill', a small vascular projection from the cavity floor immediately beneath the posterior extremity of the genital duct in the Neritidae (e.g. Fretter, 1965: fig. 1c).

Both *Pisulina* and *Neritilia* have the hypobranchial gland on the right pallial roof (Figs 1B, 4A, B, 15B: rhg; Andrews, 1937). In *Pisulina* it lies between the right shell muscle and the genital duct, extending from the back of the mantle cavity anteriorly to the mouth of the cavity. The gland in *Neritilia rubida*

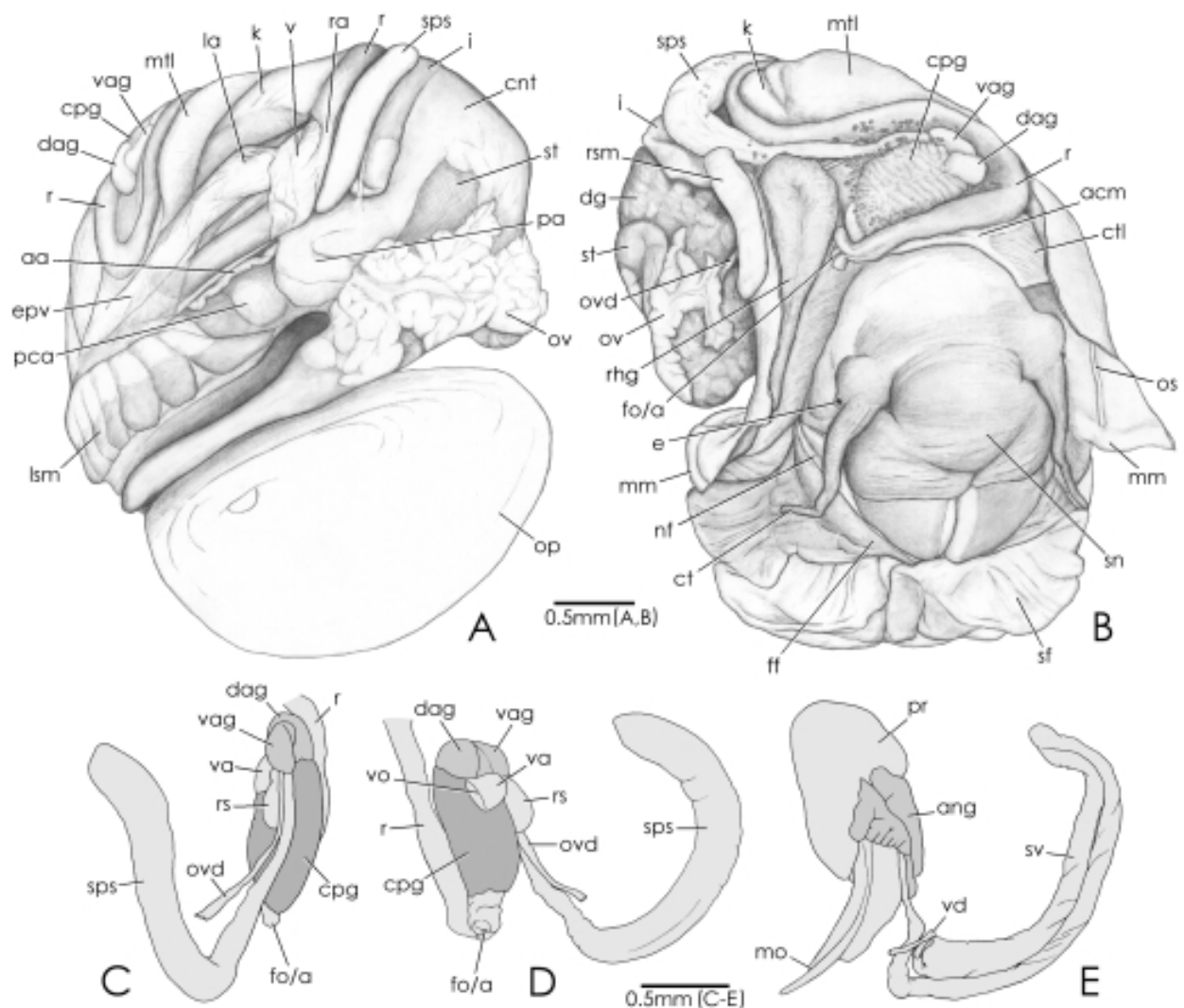


**Figure 13.** Sagittal section of closed neritid eye (*Smaragdia rangiana*) with vitreous body, cornea and eye stalk. Haematoxylin and eosin. Scale bar = 50  $\mu$ m. Abbreviations: co, cornea; es, eye stalk; re, retina; vi, vitreous mass.

further expands over the floor and the posterior wall of the mantle cavity. This hypobranchial gland differs histologically from that of neritids, irrespective of its similar position and arrangement; it is devoid of supporting cells and composed of single-type, eosin-staining columnar cells, while the latter is comprised of supporting cells and two types of glandular cells (Fretter, 1965). Andrews (1937) assumed that the secretion primarily aids the passage of spermatophores from male to female in *Neritilia*, but we confirmed that the gland is well devel-



**Figure 14.** SEM shots of *Neritilia rubida* from Palau. **A.** Frontal view of right, open-pit eye. Scale bar = 50  $\mu$ m. **B.** Removed terminal oviduct and rectum. Note that female opening and anus are united to form single aperture. Scale bar = 100  $\mu$ m. Abbreviations: a: anus; cpg, capsule gland; ct, cephalic tentacle; e, eye; fo, female opening of gonoduct; r, rectum.



**Figure 15.** *Neritilia rubida* from Palau. **A, B.** Contracted female specimen from ventral (**A**) and dorsal (**B**) sides, shell and most of mantle are removed. **C, D.** Female gonoduct from posterior (**C**) and ventral (**D**) sides. **E.** Male gonoduct from dorsal side. Abbreviations: a, anus; aa, anterior aorta; acm, afferent ctenidial membrane; ang, annex gland; cpq, capsule gland; ct, cephalic tentacle; ctf, ctenidial leaflet; cnt, connective tissue beneath mantle; dag, dorsal albumen gland; dg, digestive gland; e, eye; epv, efferent pallial vein; ff, female flap; fo, female opening of gonoduct; i, intestine; k, kidney; la, left auricle; lsm, left shell muscle; mm, mantle margin; mo, male opening of gonoduct; mtl, mantle; nf, neck furrow to female flap; op, operculum; os, osphradium; ov, ovary; ovd, oviduct; pa, posterior aorta; pca, posterior cartilage of odontophore; pr, prostate gland; r, rectum; ra, right auricle; rhg, right hypobranchial gland; rs, seminal receptacle; rsm, right shell muscle; sf, sole of foot; sn, snout; sps, spermatophore sac; st, stomach; sv, seminal vesicle; va, vagina; vag, ventral albumen gland; vd, vas deferens; vo, vaginal opening.

oped in the very young, immature individuals of both *Pisulina* and *Neritilia*.

The paired oesophageal glands of *Pisulina* are much more complicated in structure than those of other Neritopsina hitherto recorded and *Neritilia* is the only other genus known to have comparable structures. Figure 5 shows the unusually long blind sacs of the anterior oesophageal glands that overlies and cover the main trunk of the anterior oesophagus. At the opening of the sac, each anterior gland is a fairly large pouch that also communicates with the posterior oesophageal gland in *Pisulina* and *Neritilia*. This posterior gland (peg) is probably an organ homologous with the gland of the same name in neritids, phenacolepadids and helicnids in Fretter (1984) and Sasaki (1998), the 'salivary gland' of neritids in Bourne (1909), and the 'oesophageal pouch' of helicnids in Bourne (1911), judging from the topological aspect. However, it differs from the glands in the other families in that it opens laterally to the oesophagus

along nearly its entire length (Fig. 5B, D; see Salvini-Plawen & Haszprunar, 1987: fig. 2b for the large, free pouch of the gland in the Neritidae). The posterior gland of neritiliids further differs from that of neritids and phenacolepadids in that its cavity lacks deep lamellae (see Fretter, 1965, 1984), and from that of helicnids in that it expands ventrally to form a trough enclosing the radular sac (see Bourne, 1911: fig. 2).

*Pisulina* and *Neritilia* also have paired floor glands in the anterior oesophagus. These are conspicuous ventral outgrowths of the oesophagus and each gland consists of two histologically distinct parts (Figs 3C, 5B). These are unknown among other neritopsines, although presumably homologous structures have been described for the Neritidae and Phenacolepadidae; the latter two families have glandular strips lateral to the median ventral ridge, which leads back from the oesophageal valve (Whitaker, 1951; Fretter, 1965, 1984), and which are similar histologically to the floor glands in *Pisulina* and *Neritilia*. The



lumina of the floor glands are continuous with grooves lateral to the median ridge and thus these glands are most probably the developed anterior tips of the glandular strips.

As briefly discussed by Kano & Kase (2000b) *Pisulina* and *Neritilia* display similar radular morphology that warrants separation of these two genera from other Neritopsina at the familial level. In the Neritiliidae the central tooth is lacking, the inner lateral tooth ('first lateral' of Schepman, 1908; Starmühlner, 1983; Kubo & Koike, 1992; 'A-central' of Baker, 1923; Russell, 1941) is longitudinally elongate and posteriorly expanded greatly to form a large lobe, and the outer lateral tooth ('D- and E-centrals' of Baker, 1923; 'E-central' of Russell, 1941; 'fourth lateral' of Starmühlner, 1983; 'fifth lateral' of Kubo & Koike, 1992) has a broad, oblique blade that bears many conspicuous cusps (e.g. Baker, 1923; Figs 6A, 8A–C). The latero-marginal plate (Fig. 8D: LMP) is another feature found only in neritiliids among the Neritopsina (all *Neritilia* species have the plate). The central tooth is also lacking in the Titiscaniidae and Neritopsidae (Fischer, 1875; Bergh, 1890; Taki, 1955), but the latter two families are totally different from *Pisulina* and *Neritilia* in the features of the lateral field.

The marginal teeth provide a good character for separating Neritiliidae from Neritidae. In *Pisulina* and *Neritilia*, all of the marginals have, below their cusps, a thin triangular prominence that is absent in the Neritidae. The prominence may be homologous with the 'angular corner' figured in the original description of *Titiscania shinkishihataii* Taki, 1955 (Titiscaniidae). *Neritopsis radula* Linnaeus, 1758 (Neritopsidae) also has the prominence, but only in the median and outer marginals. No neritopsine has the prominence other than the Neritiliidae, Titiscaniidae and Neritopsidae.

The length of the radular sac seems to be partly related to feeding strategies, but it does not entirely account for the patterns of the length seen in gastropods (Ponder & Lindberg, 1997: 143). The radular sac of *Pisulina* is extremely long (more than twice as long as the shell diameter) and loops four times (Fig. 5F). The sac is also very long and loops in *Neritilia*, as well as in *Titiscania* and *Neritopsis* (Fischer, 1875; Bergh, 1890). In contrast, it is short and nearly straight in phenacolepadids, heliciniids and most of neritids (e.g. Bourne, 1909, 1911; Baker, 1925; Starmühlner, 1969; Fretter, 1984; Beck, 1992).

The stomach of *Pisulina* shows a highly modified neritid plan; it has an unusual connection with the digestive gland by a single, large orifice adjacent to the opening of the oesophagus (Fig. 7A, B). *Neritilia* also has this unusual condition, while the Neritidae, Phenacolepadidae and Helicinidae have two or more openings of the digestive gland ducts near the sorting area in the stomach (e.g. Bourne, 1909, 1911; Baker, 1925; Fretter, 1965, 1984; Sasaki, 1998).

The rectum is strongly curved on the pallial roof in *Pisulina* and *Neritilia* (Figs 4B, C, 7A, 15A, B: r; Andrews, 1937). Due to this curve, the ctenidium loses its direct connection with the kidney and left auricle, and the pallial gonoduct, extending along the rectum, produces a right or acute angle in the middle of its course (Figs 4B, C, 11, 15B–E). In addition, the anus of the two genera opens in the depths of the pallial cavity on the right. In the Neritidae the rectum is weakly curved or nearly straight on the pallial roof left of the gonoduct and terminates as an anus close to the mantle margin (e.g. Bourne, 1909; Fretter, 1965; Sasaki, 1998).

The single left kidney is simple in *Pisulina* and *Neritilia*, compared with those of the Neritidae, Phenacolepadidae and Helicinidae. The kidney consists of glandular and non-glandular portions in the latter three families (Bourne, 1909, 1911; Starmühlner, 1969; Fretter, 1984; Sasaki, 1998; Estabrooks, Kay & McCarthy, 1999). The glandular portion is filled with folded walls, connected with the pericardium by the renopericardial duct and opens posteriorly into the non-glandular

portion. The non-glandular portion (bladder) is a large smooth area and opens to the pallial cavity. In contrast, the kidney in the Neritiliidae is not divided into two parts, and is represented merely by a large internal cavity that is glandular throughout and has weak folds on the roof (Fig. 9B).

In *Pisulina* the visceral nerve loop is incomplete due to the lack of the supraoesophageal connective, and the supra- and suboesophageal and right pleural ganglia have been fused together to form a single ganglion. Similar nerve configuration is known in *Theodoxus* of the Neritidae (Bourne, 1909) and in *Phenacolepas* (Fretter, 1984: fig. 6), as well as in *Neritilia*. In most neritid genera, on the other hand, the loop is complete and linked with a thin supraoesophageal connective and the suboesophageal ganglion comes in contact, but does not fuse with, the right pleural ganglion (Bourne, 1909; Starmühlner, 1969; Sasaki, 1998). According to Sasaki (1998) the genus *Waldemaria* (Helicinidae) retains the supraoesophageal connective, while Bourne (1911) showed that this connective is absent in all heliciniid species he studied. The incomplete visceral loop may partly represent phylogenetic affinity among the above taxa, but it is most probable that the modification of the nervous system to give more direct pathways has occurred repeatedly among the Neritopsina in connection with reduction in body size.

*Pisulina* also shares a number of unique features of the reproductive system with *Neritilia* (Figs 11, 15). Andrews (1937) briefly described the reproductive system of *Neritilia succinea* as the simplest among the 'Neritidae', and mentioned that the 'Neritiliinae' would represent the lowest subdivision of the family. Discussed below are the common characteristics of the reproductive system in the neritiliid genera.

The spermatophore sac in females and seminal vesicle in males are conspicuous features shared between *Pisulina* and *Neritilia*. The spermatophore sac (sps) is very long and club-shaped, passing back alongside the rectum and projecting into the pericardium. The seminal vesicle (sv) is double, coils only slightly and occupies the same position as the spermatophore sac (Figs 1B, 4, 11, 15; Andrews, 1937). Starmühlner (1976) erroneously figured the doubled seminal vesicle as a spermatophore sac for *Neritilia rubida*.

The doubled seminal vesicle in the Neritiliidae is quite different from the highly convoluted nature of the same organ in all the other Neritopsina (Bourne, 1909; Berry *et al.*, 1973; Sasaki, 1998), other than some helicinioids (Baker, 1925; Thompson, 1980). Houston (1990) figured a simple straight duct for the seminal vesicle of *Titiscania limacina* Bergh, 1890, but in our dissections of that species, we found that the vesicle is convoluted in all mature males. The vas deferens of *Pisulina* and *Neritilia* further differs from that of Neritidae in the absence of the 'epididymis', a coiled, closely packed and black-pigmented part preceding the seminal vesicle (e.g. Bourne, 1909).

The vaginal opening in *Pisulina* and *Neritilia* forms a transverse slit situated far from the female opening and anus, ventral to the albumen gland (Figs 11A, B, 12, 15D). Andrews' (1937) description of the location of the vaginal opening is incorrect and Starmühlner (1976, 1983, 1988, 1993) erroneously reported that three species of the genus have only one female gonopore. Among other neritopsines, the Helicinoidea alone have a posteriorly positioned vaginal opening (e.g. Bourne, 1911; Thompson, 1980). The vaginal opening of the Neritidae and Phenacolepadidae is positioned at the right of the pallial cavity near the mantle margin, together with the female opening and anus (e.g. Andrews, 1937; Fretter, 1984).

The Neritiliidae are unique in their arrangement of the seminal receptacle. The seminal receptacle of the Neritidae and Phenacolepadidae diverges from the 'duct of the seminal receptacle' (vaginal canal), which connects the spermatophore sac and dorsal albumen gland (Bourne, 1909; Starmühlner, 1969; Berry *et al.*, 1973; Sasaki, 1998). In neritiliids there is no

independent duct from the seminal receptacle to the albumen gland and the sperm from the receptacle enter the gland via the vagina (Figs 11A, B, 12, 15C, D).

*Pisulina* and *Neritilia* have no crystal (reinforcement) sac. This sac is found only in the Neritidae, where it lies to the left of the capsule gland and opens together with the gland as a female opening. In *Nerita* it is full of spheroidal calcareous granules produced in the digestive gland (Andrews, 1935; see SEM micrographs in Bandel, 1990: fig. 9E, F; and Sasaki, 1998: fig. 77h). In *Neritina*, *Septaria*, *Theodoxus* and *Neritodryas*, on the other hand, the sac contains sand grains gathered with the food and the anus adjoins the female opening to pass the solids in the faeces into the crystal sac (e.g. Andrews, 1937). Both the secreted granules and sand reinforce the egg capsules (e.g. Andrews, 1933, 1935). *Smaragdia* is the only genus devoid of the sac in the Neritidae. Andrews (1937) reported a crystal sac in *Smaragdia viridis*, but might have confused the vestigial spermatophore sac with it. Starmühlner (1976, 1983, 1988, 1993) erroneously figured crystal sacs in three *Neritilia* species, although Andrews (1937) precisely described that the egg capsule of *Neritilia succinea* is reinforced at the mouth of the capsule gland by sand directly passed from the anus. In *N. radula* and *N. succinea* the female opening and anus are united into a single aperture (Figs 14B, 15D). *Pisulina* may lay an egg capsule that contains only one embryo as in *Neritilia*, judging from the similarities in the female reproductive tract and protoconch size and morphology (Kano & Kase 2000b). The egg capsule may also be reinforced by sand, as are the capsules of some species of *Neritilia* with separate female opening and anus (as in *Pisulina*; Kano, Sasaki & Ishikawa, 2001).

#### Phylogeny and classification

The classification of the superorder Neritopsina is still far from a phylogenetic one (Haszprunar, 1988b: 412). A number of anatomical studies of the superorder have mostly been limited to the Neritidae, Helicinidae and Phenacolepadidae. No anatomical account of *Neritopsis*, the sole extant genus of the Neritopsidae that is a very old offshoot back to the Silurian (Tracey, Todd & Erwin, 1993), has been published, aside from a brief note on the external morphology by Fischer (1875). The shell-less slug *Titiscania* (Titiscaniidae) and the minute terrestrial Hydrocenidae have been poorly studied (Bergh, 1890; Thiele, 1910; Marcus & Marcus, 1967). The detailed systematic position of *Pisulina* should ultimately be determined cladistically, but at present the lack of sufficient knowledge of the soft parts in other taxa prevents such an analysis. It is reasonable, however, to conclude at present that *Pisulina* is not a member of the Neritidae, but should be allocated to the Neritiliidae. The subfamily Smaragdiinae of the Neritidae, to which *Pisulina* was previously assigned (e.g. Wenz, 1938; Knight *et al.*, 1960), is evidently not a monophyletic group. *Smaragdia* shares the well-developed opercular fold of the metapodium with *Pisulina*, but it is the only anatomical feature restricted to the two genera. Taking the great disparity in many other anatomical traits into consideration, the homology of the developed opercular folds in the two can readily be rejected.

The close relationship between *Pisulina* and *Neritilia*, and independence of the family Neritiliidae, are supported by 19 conchological and anatomical features shared between these two genera: (1) an inclined protoconch; (2) spiral ridges on the larval shell; (3) almost perpendicularly arranged prisms of the outer shell layer (Kano & Kase, 2000b); (4) open-pit eyes; (5) female flap on the foot with a ciliated neck furrow from the mantle cavity; (6) caeca on the visceral mass and corresponding shell pores; (7) reduced left ctenidium; (8) right hypobranchial gland composed of single-type cells; (9) two complicated pairs of oesophageal glands; (10) another pair of ovoid glands in the

oesophageal floor; (11) inner lateral tooth of radula with a largely expanded lobe; (12) elongated outer lateral tooth with a oblique, come-like blade; (13) a lateromarginal plate; (14) single large aperture of the digestive gland into the stomach; (15) strongly curved rectum on the pallial roof; (16) transverse slit-shaped vaginal opening situated far back from the female opening and anus; (17) absence of independent duct from the seminal receptacle to the albumen gland; (18) very long, club-shaped spermatophore sac extending to the pericardium; and (19) doubled seminal vesicle. These features are all restricted to the Neritiliidae among the Neritopsina and are possible synapomorphies of the family. Some of them, including the shell pores and lateromarginal plate, are also found in non-neritopsine gastropods, but it is unlikely that these are plesiomorphies or that the Neritiliidae are basal neritopsine (the Neritopsidae is the first offshoot; Holthuis, 1995; Y. Kano, S. Chiba & T. Kase, unpublished data).

The genus *Neritilia* includes more than a dozen extant species (only six of which have been described) that inhabit freshwater streams and anchialine waters (bodies of haline water with more or less extensive subterranean connection to the sea, and showing noticeable marine, as well as terrestrial influences; Stock, Iliffe & Williams, 1986) in tropical and subtropical regions. Schepman (1908) established the monotypic family Neritiliidae for *Neritilia*, and diagnosed it by its unique radular morphology and concentrically-growing operculum. He stated that '*Neritilia* has as much or even more right to stand as a separate family than *Neritopsis*', and 'Its place may be near some genus of operculate landshells' (i.e. the Helicinoidea or Hydrocenidae). However, the genus was later classified as a subfamilial taxon within the Neritidae (e.g. Baker, 1923), presumably due to the overall similarity to neritids in the shape of the teleoconch. Andrews (1937) was the first author to note the primitiveness of *Neritilia* in the female genital system, but he maintained the genus within the Neritidae. Holthuis (1995) has recently shown the paraphyly of 'Neritidae' (*sensu* Thiele, 1929; Wenz, 1938; Knight *et al.*, 1960) and concluded that *Neritilia* is the sister group of the Neritidae + Phenacolepadidae. The paraphyly of the 'Neritidae' and the monophyly of *Pisulina* + *Neritilia* are supported by a molecular analysis using 28S rRNA sequences (Y. Kano *et al.*, unpublished data). In addition to these two genera, Neritiliidae include the Miocene genus *Pisulinella* (Kano & Kase, 2000a), and several undescribed genera from anchialine and submarine-cave waters (Y. Kano, unpublished).

*Pisulina* is distinguishable from all the *Neritilia* species hitherto described in the following features: (1) absence of an additional layer in the planktotrophic larval shell (Kano & Kase, 2000b); (2) presence of the apertural projection or teeth on the teleoconch inner lip; (3) absence of the 'very small list (protuberance) inside the aperture' (Schepman, 1908) acting as the innermost stop of the operculum in *Neritilia*; (4) paucispiral opercular formation (concentric in *Neritilia*); (5) opercular apophysis arranged parallel to the opercular surface (perpendicular to the surface in *Neritilia*); (6) presence of the opercular fold; (7) presence of the vestigial penis; (8) absence of the anterior incision of the oral disk (present in *Neritilia*; see Kubo & Koike, 1992: fig. 1c); (9) less developed hypobranchial gland on the right pallial roof; (10) much longer shell muscles; and (11) absence of their subdivision into bundles (present in the left one of *Neritilia*: Fig. 15A); (12) four loops of the radular sac (less than three in *Neritilia*); and (13) longitudinally flattened inner marginal teeth of the radula with two cusps (broad with four to 10 cusps in *Neritilia*: e.g. Baker, 1923; Kubo & Koike, 1992).

#### Regressive evolution of eyes in cryptic habitats

One of the most exciting discoveries in this study is the presence of the open-pit eye without a vitreous body in *Pisulina* and

*Neritilia* (Figs 3A, 14A); this type of eye is hitherto unknown in the subclass Orthogastropoda. Among the Gastropoda the open-pit eye has been known only in the Patellogastropoda (subclass Eogastropoda) and regarded as a plesiomorphic condition because the primitive cephalopod *Nautilus* has a similar 'pin-hole camera' type of eye (Ponder & Lindberg, 1997). The eye of the Orthogastropoda bears a vitreous body (lens) and its presence has been regarded as a synapomorphy of the subclass (Ponder & Lindberg, 1997). The majority of orthogastropod eyes are known to be closed with a cornea, while in some vetigastropod families (i.e. Pleurotomariidae, Haliotidae, Trochidae and Turbinidae) the eyes remain narrowly open but have a vitreous body (Hyman, 1967; Ponder & Lindberg, 1997; Sasaki, 1998). Taking the remote relationship between the Neritopsina and Patellogastropoda (Ponder & Lindberg, 1997; Sasaki, 1998) into consideration, the open pit eye in *Pisulina* and *Neritilia* is unlikely to be homologous with that of the latter group. Rather, it may have secondarily degenerated from the closed eye of the other Neritopsina (Fig. 13; Starmühlner, 1969: fig. 48). The closed condition of the eyes in *Neritopsis* (the first offshoot of the superorder; Holthuis, 1995) further supports the independent origin of the neritiliid open-pit eyes (Y. Kano, unpublished data). If the eyes in *Pisulina* and *Neritilia* are apomorphic and degenerative in origin, it is highly probable that the loss of the cornea and vitreous body took place in a common ancestral species that lived in gloomy to totally dark, cryptic habitats.

Degeneration of eyes has been reported in various gastropod taxa that inhabit terrestrial caves, groundwaters, interstices of marine sand and deep-sea habitats, including hydrothermal vents. The degeneration is achieved in several different ways, such as reduction in size, loss of the pigmented retina and sinking under the skin; the eyes are sometimes lost completely (Vandel, 1965; Swedmark, 1968; Zharkova, 1978; Haszprunar, 1988a, 1989; Warén & Bouchet, 1993). The degeneration in neritiliids is similar to that of the deep-sea Cocculinidae (Cocculiniformia). The eye of these limpets is a small dimple with several large bottle-shaped mucous cells that form a distinct 'basitentacular gland' (Haszprunar, 1987; Strong & Harasewych, 1999).

A troglitic life for the common neritiliid ancestor is also supported by the retention of exceptionally long cephalic tentacles in *Neritilia*. Even in the species found on the upper surface of rubble in epigeal freshwater streams, the tentacle attains a length nearly equal to or much longer than the shell diameter, while that of neritids found in such habitats is less than half the shell diameter in length. The elongation of appendages bearing sense organs is prevalent among cavernicolous animals, compensating for the loss of vision (Vandel, 1965; Culver, 1982; Langecker, 2000). Among the Neritopsina, such long tentacles are also known in *Phenacolepas*, a genus living exclusively in intertidal to subtidal, subterranean habitats (under deeply embedded rocks; Fretter, 1984; Ponder, 1998; Warén & Bouchet, 2001). The unpigmented skin of some *Neritilia* species (Kano *et al.*, 2001), as in *Pisulina*, is additional evidence for the cave life of the common ancestor. Unpigmented skin is also widespread among cavernicolous animals (Culver, 1982; Langecker, 2000).

The retina of *Patella* (Patellogastropoda) is composed of a single type of cells (Marshall & Hodgson, 1990), while it is of two types (pigmented and sensory) in cephalopods (excluding *Nautilus*) and orthogastropods (Ponder & Lindberg, 1997). A better understanding of the ultrastructure of the neritiliid open eye will require TEM observations in future studies.

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